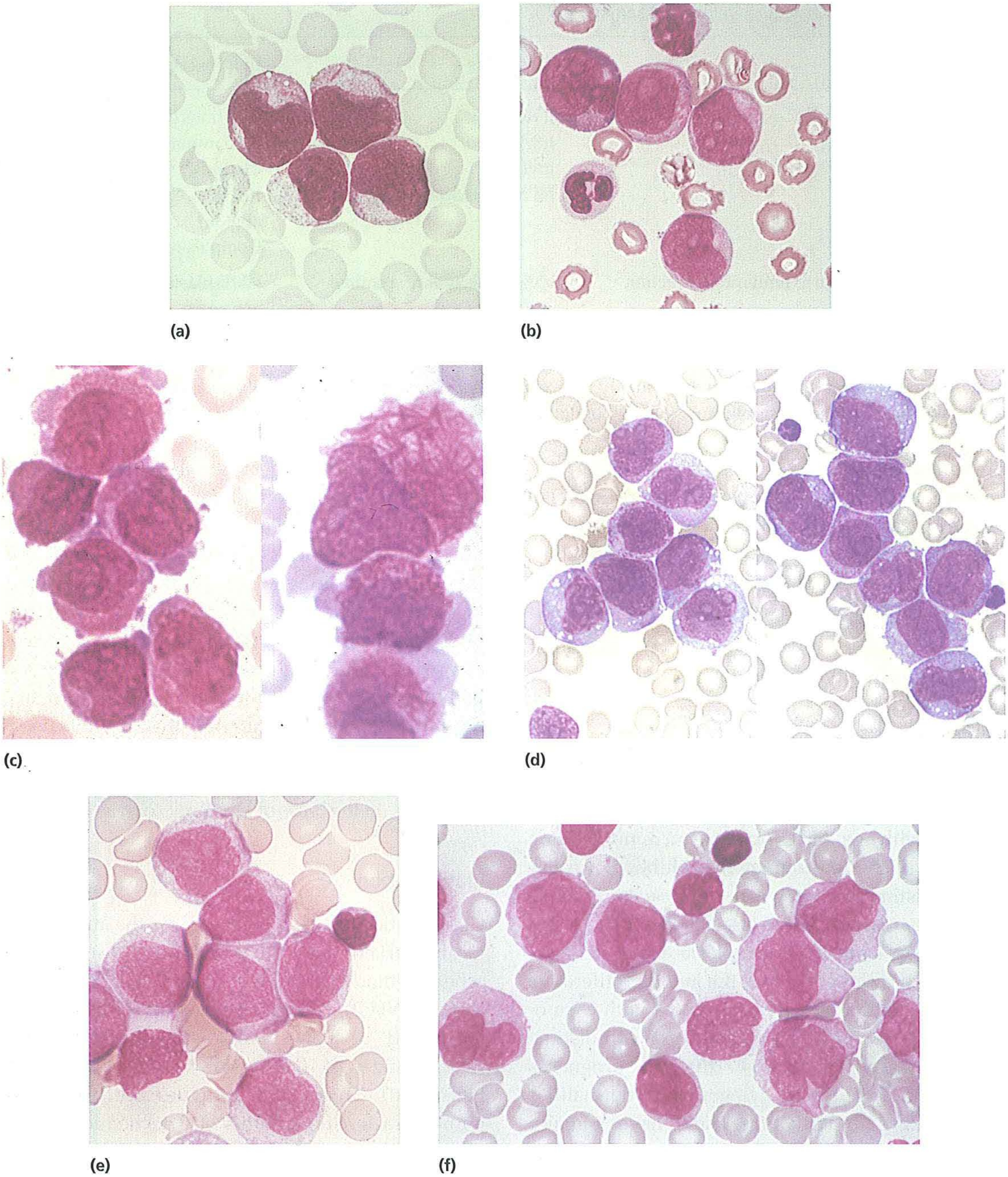
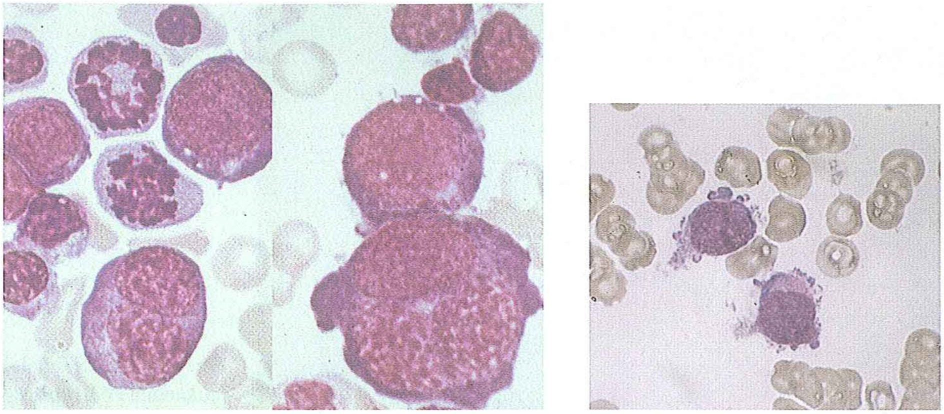


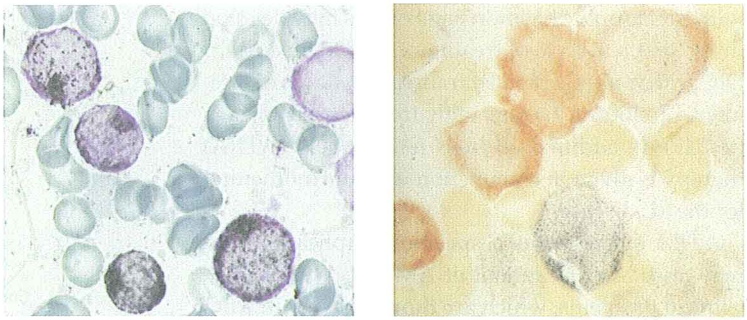
Fig. 12.10 The French–American–British (FAB) classification of acute myeloid leukaemia. **(a)** M_1 blast cells show few granules but may show Auer rods, as in this case; **(b)** M_2 cells show multiple cytoplasmic granules; **(c)** M_3 blast cells contain prominent granules or multiple Auer rods; **(d)** M_4 blasts have some monocytoid differentiation; **(e)** M_{5a}—monoblastic leukaemia in which >80% of blasts are monoblasts; **(f)** M_{5b}—monocytic but <80% of blasts are monoblasts.

(Continued)



(g) (h)

Fig. 12.10 (*Continued*) **(g)** M_6 showing preponderance of erythroblasts; **(h)** M_7—megakaryoblastic leukaemia showing cytoplasmic blebs on blasts.



(a) (b)

Fig. 12.11 Cytochemical staining in acute myeloid leukaemia. **(a)** Sudan black B shows black staining in the cytoplasm. **(b)** M_4 (myelomonocytic): non-specific esterase/chloracetate staining shows orange-staining monoblast cytoplasm and blue-staining (myeloblast) cytoplasm.

valid as generally their treatment and prognosis are similar. However, differences in treatment according to subtype are being introduced.

Clinical features

Clinical features resemble those of ALL. Anaemia and thrombocytopenia are often profound. A bleeding tendency caused by thrombocytopenia and disseminated intravascular coagulation (DIC) is characteristic of the M_3 variant of AML. Tumour cells can infiltrate a variety of tissues. Gum hypertrophy and infiltration (Fig. 12.12), skin involvement and CNS disease are characteristic of the myelomonocytic (M_4) and monocytic (M_5) types. An isolated mass of leukaemic blasts is usually referred to as a granulocytic sarcoma.

Investigations

The general haematological and biochemical findings are similar to those seen in ALL. Tests for DIC are often positive in patients with the promyelocytic (M_3) variant of AML. As explained earlier, cytogeneic and molecular analysis is critical for determining the prognosis and developing a treatment plan (Table 12.6).

Treatment

Management is both supportive and specific.

1 Supportive treatment is based on the same principles as that for ALL. Problems unique to AML include the haemorrhagic syndrome associated with the AML M_3 variant. The disease may present

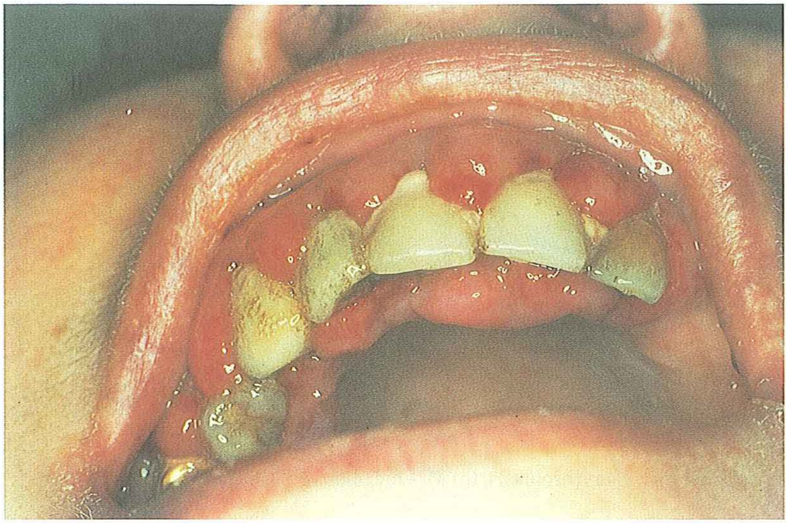


Fig. 12.12 Acute myeloid leukaemia FAB type M_5 (monocytic): the gums are swollen and haemorrhagic because of infiltration by leukaemic cells.

with catastrophic haemorrhage or this may develop in the first few days of treatment. It is treated as for DIC with multiple platelet transfusions and replacement of clotting factors with fresh frozen plasma (p. 349). In addition all-*trans* retinoic acid (ATRA) therapy is given in conjunction with chemotherapy for the M_3 disease.

ATRA syndrome is a specific complication that may arise after treatment of AML-M_3 with ATRA. Clinical problems, which are thought to result from the neutrophilia that follows differentiation of promyelocytes from the bone marrow (p. 138), include fever, hypoxia with pulmonary infiltrates and fluid overload. Treatment is with dexamethasone 10 mg intravenously twice daily and ATRA is only discontinued in very severe cases.

2 Specific therapy of AML is primarily with the use of intensive chemotherapy. This is usually given in four blocks each of approximately 1 week and the most commonly used drugs include cytosine arabinoside (in conventional or high doses), daunorubicin, idarubicin, mitoxantrone and etoposide (Fig. 12.13). All the AML subtypes (FAB M_0–M_7) are treated similarly except for the promyelocytic (M_3) variant associated with the t(15; 17) translocation in which ATRA is added to the initial chemotherapy. A typical good response in AML to cytotoxics is shown in Fig. 12.14. The drugs are myelotoxic with limited selectivity between leukaemic and normal marrow cells and so marrow failure resulting from the chemotherapy is severe, and prolonged and intensive supportive care is required. Maintenance therapy is of no value except in AML M_3 and CNS prophylaxis is not usually given in AML.

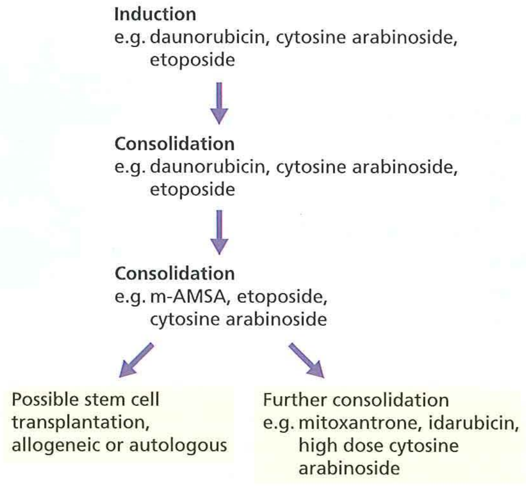


Fig. 12.13 Acute myeloid leukaemia: flow chart illustrating typical treatment regimen.

An important concept developing in AML therapy is that of basing the treatment schedule of individual patients on their risk group. Favourable cytogenetics and remission after one course of chemotherapy both predict for a better prognosis. In contrast, monosomy 5 or 7 abnormalities, blast cells

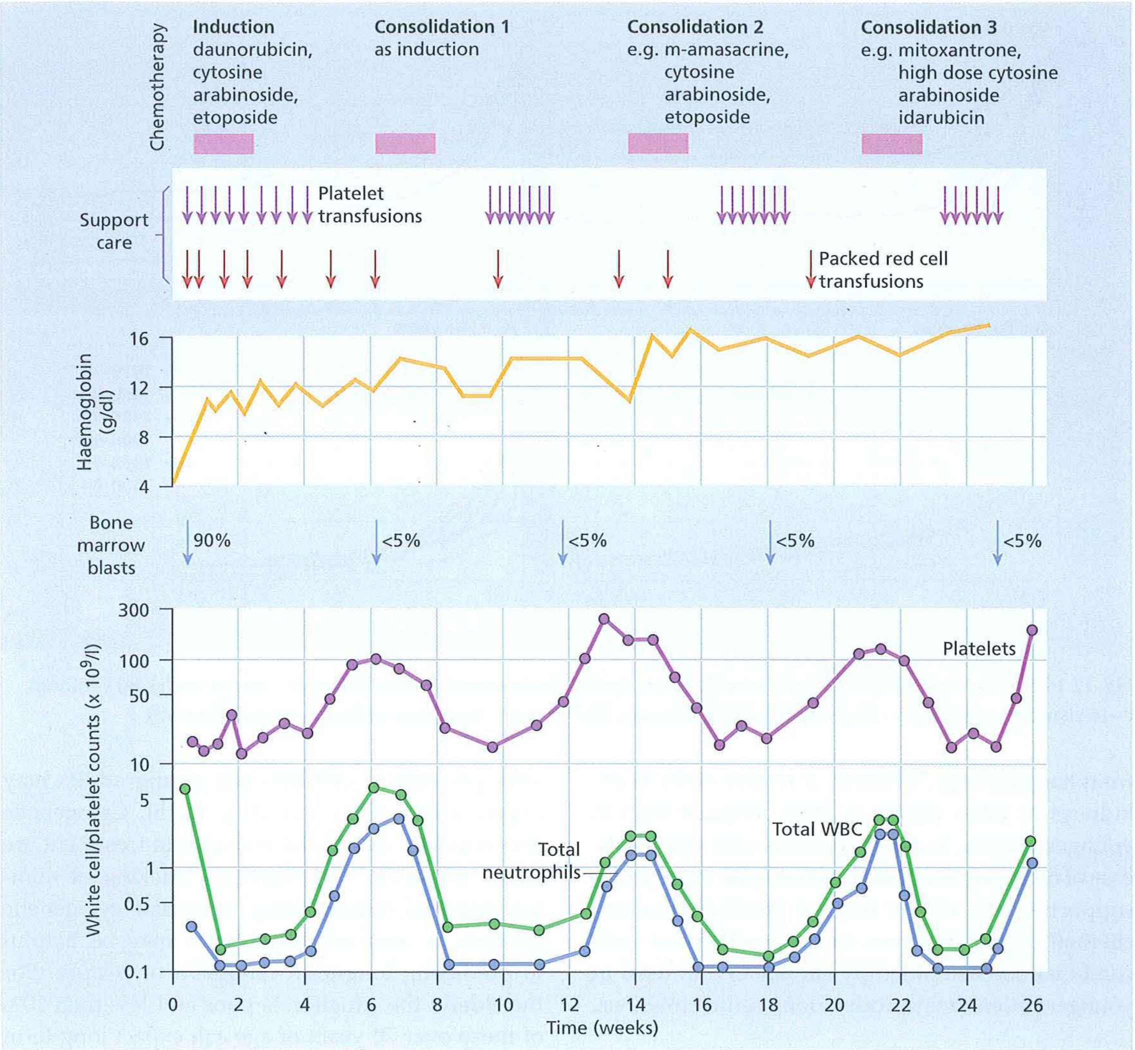


Fig. 12.14 Typical flow chart for the management with chemotherapy of acute myeloid leukaemia.

with *Flt-3* mutations or poorly responsive disease places patients into poor risk groups which may need more intensive treatments (Table 12.6). Monoclonal immunoconjugates targeted against CD33 (e.g. Mylotarg) or CD45 provide an additional therapeutic option for AML therapy.

Stem cell transplantation

Autologous transplantation reduces the rate of relapse but adds further toxicity to the treatment regime and is of no overall benefit. Allogeneic stem cell transplantation (SCT) is used in some centres in patients under 65 years old with a human leucocyte antigen (HLA) matching sibling donor with standard or poor risk AML in first remission, although other groups save it as an option for relapsed disease. Patients with t(8; 21), t(15; 17) and inv16 who go into remission after the first course do not have SCT unless they subsequently relapse.

Patients over 70 years of age

Results of AML therapy in the elderly are poor because of primary disease resistance and poor tolerability of intensive treatment protocols. Death

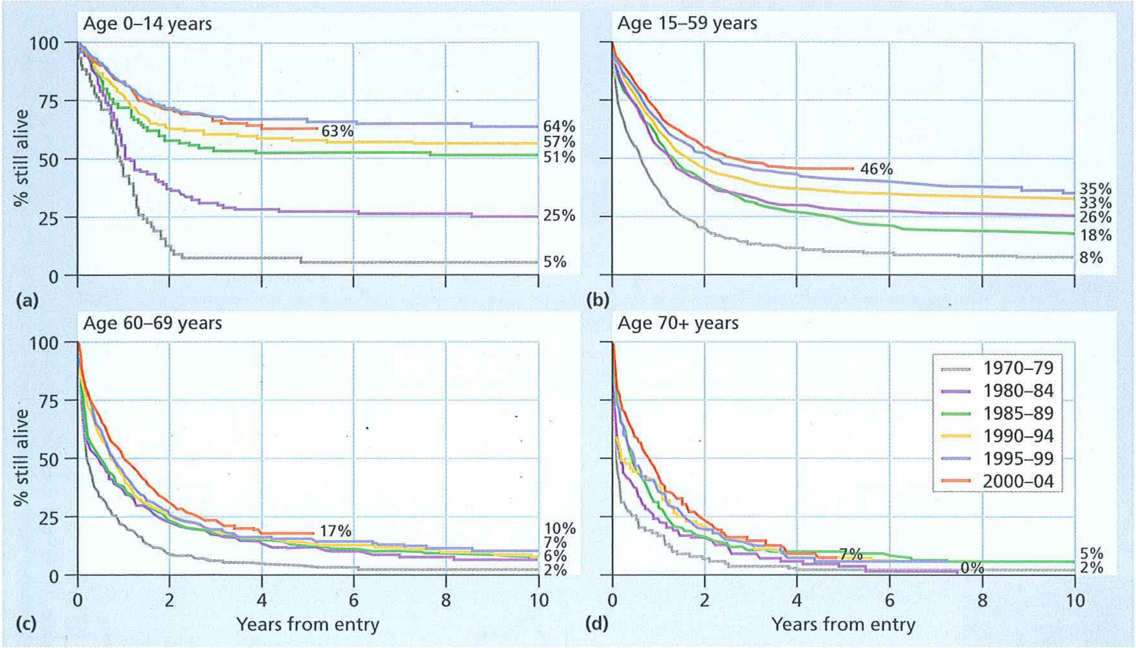


Fig. 12.15 Survival in children and adults with acute myeloid leukaemia (Medical Research Council trials). **(a)** Children 0–14 years; **(b)** patients 15–59 years; **(c)** age 60–69 years; **(d)** 70+ years. (Courtesy of Professor A.K. Burnett)

from haemorrhage, infection or failure of the heart, kidneys or other organs is more frequent than in younger patients. In elderly patients with serious disease of other organs, the decision may be made to use supportive care with or without gentle single-drug chemotherapy. However, in those otherwise well, combination chemotherapy similar to that used in younger patients may produce long-term remissions.

Treatment of relapse

Most patients suffer relapse of their disease and the outlook will depend on age, the duration of the first remission and the cytogenetic risk group. In addition to further chemotherapy, allogeneic SCT is usually performed in those patients who can tolerate the procedure and who have a suitable HLA matching related or unrelated donor. Arsenic trioxide is useful in management of relapsed M_3 variant.

Prognosis

The prognosis for patients with AML has been improving steadily, particularly for younger patients and most recently for the 60–70-year-old group. Fifty per cent of children and young adults may expect a long-term cure (Fig. 12.15). Cytogenetic abnormalities and initial response to treatment are major predictors of prognosis. Tracking of minimal residual disease using molecular cytogenetic markers or aberrant phenotypes may be helpful in predicting long-term remission or relapse. For the elderly the situation is poor and less than 10% of those over 70 years of age can expect long-term remission.

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CHAPTER 13

13 Chronic myeloid leukaemia

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The chronic leukaemias are distinguished from acute leukaemias by their slower progression. Paradoxically, they are also more difficult to cure. Chronic leukaemias can be broadly subdivided into myeloid (Table 13.1) and lymphoid groups (Chapter 15).

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a clonal disorder of a pluripotent stem cell. The disease accounts for around 15% of leukaemias and may occur at any age. The diagnosis of CML is rarely difficult and is assisted by the characteristic presence of the Ph chromosome (Fig. 13.1d). This results from the t(9; 22) (q34; q11) translocation between chromosomes 9 and 22 as a result of which part of the proto-oncogene c-*ABL* is moved to the *BCR* gene on chromosome 22 (Fig. 13.1a) and part of chromosome 22 moves to chromosome 9. The abnormal chromosome 22 is the Ph chromosome (Fig. 13.1d). In the Ph translocation 5′ exons of *BCR* are fused to the 3′ exons of *ABL* (Fig. 13.1b,c). The resulting chimeric *BCR-ABL* gene codes for a fusion protein of size 210 kDa (p210). This has tyrosine kinase activity in excess of the normal 145-kDa ABL product. The Ph translocation is also seen in a minority of cases of acute lymphoblastic leukaemia (ALL) and in some of these the breakpoint in *BCR* occurs in the same region as in CML. However, in other cases the breakpoint in *BCR* is further upstream, in the intron between the first and second exons, leaving only the first *BCR* exon intact. This chimeric *BCR-ABL* gene is expressed as a p190 protein which, like p210, has enhanced tyrosine kinase activity. In a minority of patients, the Ph abnormality cannot be seen by microscopic karyotypic analysis but the same molecular rearrangement is detectable by more sensitive techniques; for example, fluorescence *in situ* hybridization (FISH) (Fig. 13.1e) or polymerase chain reaction (PCR) (see p. 90). This Ph-negative *BCR-ABL*-positive CML behaves clinically like Ph-positive CML. As the Ph chromosome is an acquired abnormality of haemopoietic stem cells it is found in cells of both the myeloid (granulocytic, erythroid and megakaryocytic) and lymphoid (B and T cell) lineages.

A great increase in total body myeloid cell mass is responsible for most of the clinical feaures. In at least 70% of patients who do not respond well to imatinib there is a terminal metamorphosis to acute leukaemia, often preceded by an accelerated phase.

Table 13.1 Classification of chronic myeloid leukaemias (CML).

Chronic myeloid leukaemia, Ph positive (CML, Ph+) (chronic granulocytic leukaemia, CGL)
Chronic myeloid leukaemia, Ph negative (CML, Ph–) (atypical)
Juvenile chronic myeloid leukaemia
Chronic neutrophilic leukaemia
Eosinophilic leukaemia
Chronic myelomonocytic leukaemia (CMML) (see myelodysplasia, p. 186)

Clinical features

This disease occurs in either sex (male : female ratio of 1.4 : 1), most frequently between the ages of 40 and 60 years. However, it may occur in children and neonates, and in the very old. In most cases there are no predisposing factors but the incidence was increased in survivors of the atom bomb exposures in Japan. Its clinical features include the following:

1 Symptoms related to hypermetabolism (e.g. weight loss, lassitude, anorexia or night sweats).

2 Splenomegaly is nearly always present and is frequently massive. In some patients, splenic enlargement is associated with considerable discomfort, pain or indigestion.

3 Features of anaemia may include pallor, dyspnoea and tachycardia.

4 Bruising, epistaxis, menorrhagia or haemorrhage from other sites because of abnormal platelet function.

5 Gout or renal impairment caused by hyperuricaemia from excessive purine breakdown may be a problem.

6 Rare symptoms include visual disturbances and priapism.

7 In up to 50% of cases the diagnosis is made incidentally from a routine blood count.

Laboratory findings

1 Leucocytosis is usually $>50 \times 10^9/L$ and sometimes $>500 \times 10^9/L$ (Fig. 13.2). A complete spectrum of myeloid cells is seen in the peripheral blood. The levels of neutrophils and myelocytes exceed those of blast cells and promyelocytes (Fig. 13.3).

2 Increased circulating basophils.

3 Normochromic, normocytic anaemia is usual.

4 Platelet count may be increased (most frequently), normal or decreased.

5 Neutrophil alkaline phosphatase score is invariably low. It is raised in the myeloproliferative diseases and infections.

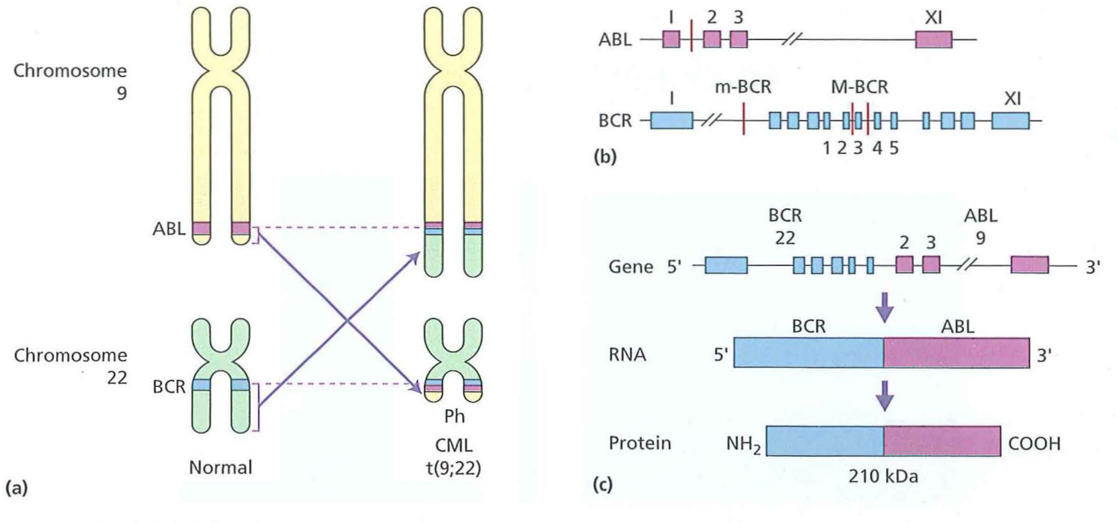
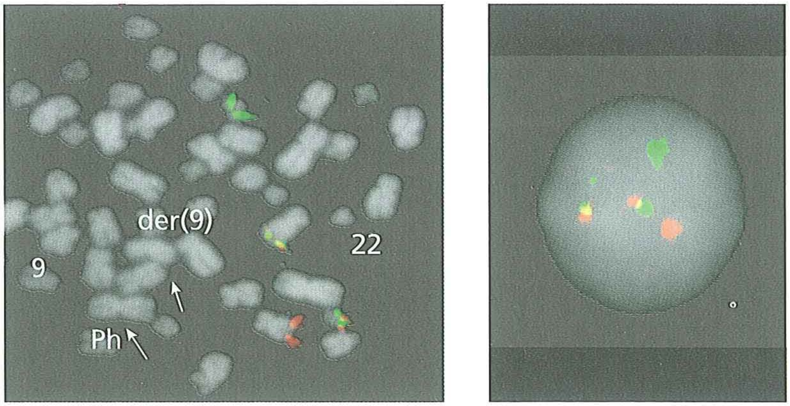


Fig. 13.1 The Philadelphia chromosome. **(a)** There is translocation of part of the long arm of chromosome 22 to the long arm of chromosome 9 and reciprocal translocation of part of the long arms of chromosome 9 to chromosome 22 (the Philadelphia chromosome). This reciprocal translocation brings most of the *ABL* gene into the *BCR* region on chromosome 22 (and part of the *BCR* gene into juxtaposition with the remaining portion of *ABL* on chromosome 9). **(b)** The breakpoint in *ABL* is between exons 1 and 2. The breakpoint in *BCR* is at one of the two points in the major breakpoint cluster region (M-BCR) in chronic myeloid leukaemia (CML) or in some cases of Ph+ acute lymphoblastic leukaemia (ALL). **(c)** This results in a 210-kDa fusion protein product derived from the *BCR-ABL* fusion gene. In other cases of Ph+ ALL, the breakpoint in *BCR* is at a minor breakpoint cluster region (m-BCR) resulting in a smaller *BCR-ABL* fusion gene and a 190-kDa protein. *(Continued)*

(d)



(i) (ii)

(e)

Fig. 13.1 (*Continued*) **(d)** Karyotype showing the t(9; 22) (q34; q11) translocation. The Ph chromosome is arrowed. **(e)** Visualization of the Philadelphia chromosome on: (i) dividing (metaphase); and (ii) quiescent (interphase) cells by fluorescence *in situ* hybridization (FISH) analysis (ABL probe in red and BCR probe in green) with fusion signals (red/green) on the Ph and der(9) chromosomes. (Courtesy of Dr Ellie Nacheva)

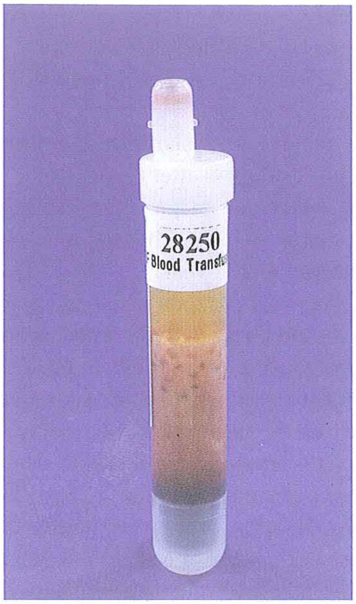


Fig. 13.2 Chronic myeloid leukaemia: peripheral blood showing a vast increase in buffy coat. The white cell count was 532×10^9/L.

6 Bone marrow is hypercellular with granulopoietic predominance.
7 Ph chromosome on cytogenetic analysis (conventional or FISH) of blood or bone marrow (Fig. 13.1).
8 Serum uric acid is usually raised.

Treatment

Treatment of chronic phase

Tyrosine kinase inhibitors Imatinib (Glivec) was designed as a specific inhibitor of the BCR-ABL fusion protein and blocks tyrosine kinase activity by competing with adenosine triphosphate (ATP) binding (Fig. 13.4). It is the first-line drug in the management of chronic phase disease. At a dose of 400 mg/day it is able to produce a complete haematological response in virtually all patients (Fig. 13.5). Side-effects include skin rash, fluid retention, muscle pains and nausea. Neutropenia and thrombocytopenia may occur and although neutropenia may be managed by granulocyte colony-stimulating factor (G-CSF) in some cases, dose reduction or cessation may be required. Imatinib is highly effective in reducing the number of tumour cells in the bone marrow and this may be monitored by karyotypic analysis of the bone marrow together with PCR analysis for BCR-ABL transcripts in marrow or blood. A complete cytogenetic response is defined as the absence of Ph-positive metaphases on cytogenetic analysis of the bone marrow. The aim is to achieve complete cytogenetic response in all patients as this is associated with improved survival. In the first year after starting treatment, blood should be studied every 3 months and the marrow

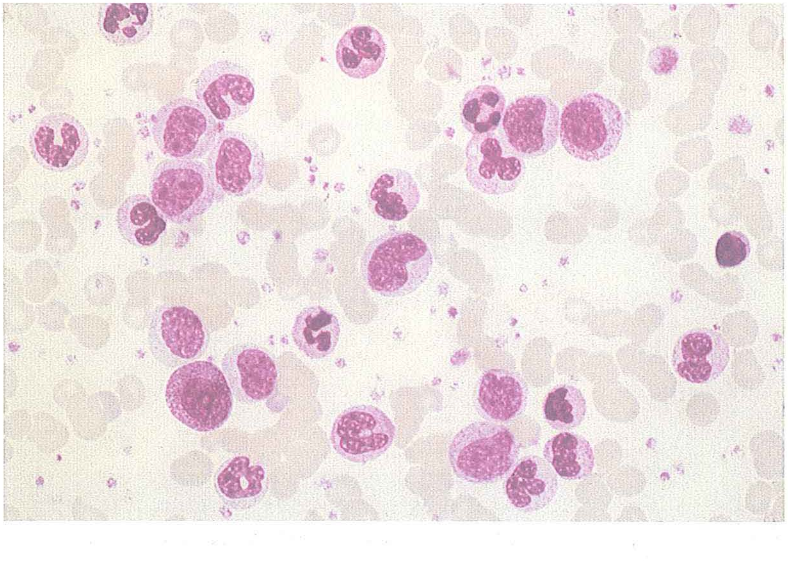


Fig. 13.3 Chronic myeloid leukaemia: peripheral blood film showing various stages of granulopoiesis including promyelocytes, myelocytes, metamyelocytes and band and segmented neutrophils.

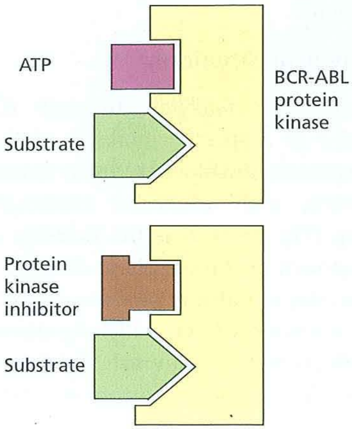


Fig. 13.4 Mode of action of the tyrosine kinase inhibitor STI-571. It blocks the adenosine triphosphate (ATP) binding site.

at 6 monthly intervals. Patients with suboptimal response can be identified as those who:

1 Fail to achieve a complete haematological response after 3 months;

2 Fail to achieve any significant cytogenetic response in bone marrow after 6 months; or

3 Fail to achieve a major cytogenetic response in bone marrow — <25% Ph+ metaphases by cytogenetic analysis after 1 year.

It is unclear how patients who have a suboptimal response should be treated. Potential options are an increase in the dose of imatinib to 600 or 800 mg/day, substitution or addition of second generation tyrosine kinase inhibitors dasatinib or nilotinib which can overcome resistance to imatinib or early allogeneic stem cell transplantation. These questions, as well as combination of imatinib with other drugs (e.g. interferon or cytosine arabinoside), are being assessed in trials. Molecular screening for mutations in the *BCR-ABL* fusion gene can be predictive for

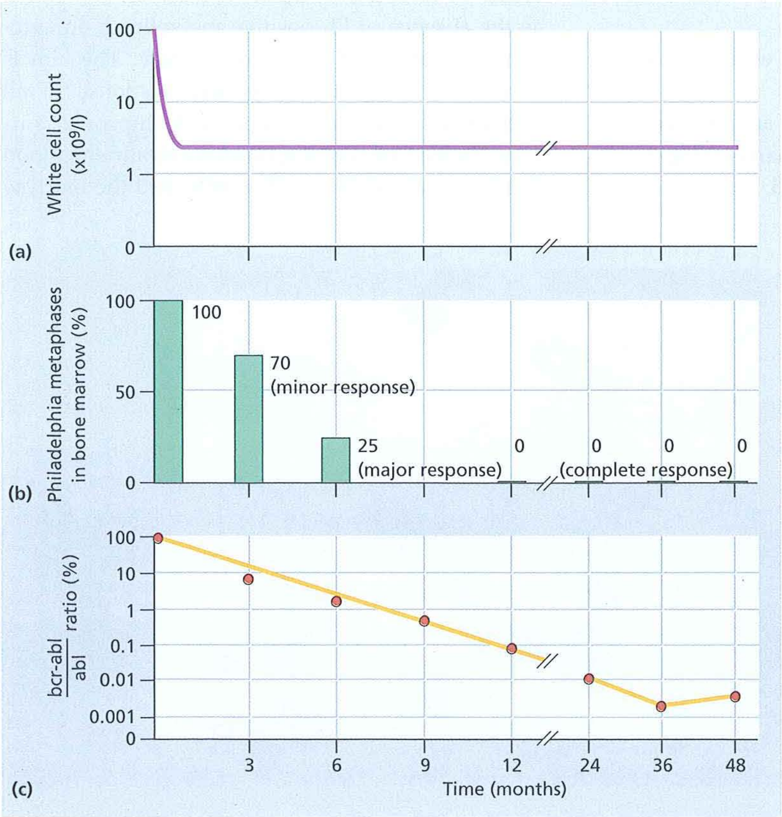


Fig. 13.5 Example of the haematological and cytogenetic response in a patient with chronic myeloid leukaemia who achieves complete remission with imatinib therapy. **(a)** The white cell count returns to normal within days. **(b)** Karyotypic examination of the bone marrow reveals a gradual reduction in the number of Philadelphia chromosomes over the first year. **(c)** Polymerase chain reaction (PCR) analysis of the bone marrow or blood shows a reduction in the number of bcr-abl transcripts in comparision with the normal abl transcript. Bcr-abl transcripts continue to be detected at a very low level but can become negative in some patients. In this case analysis was performed on bone marrow for the first 12 months and on peripheral blood thereafter.

acquired resistance to imatinib in patients in whom there is an increase in bcr-abl transcripts.

It is not yet known if patients may stop imatinib if they become negative for *BRC-ABL* transcripts by PCR analysis. It is possible that imatinib, and similar drugs in development, may prove to be a cure for some patients with CML but this will need much longer clinical follow-up than is available at the present time.

Allopurinol is used in the initial phase of treatment to prevent hyperuricaemia and gout.

Second-line drugs for patients unable to take imatinib or have a poor response to it are discussed below.

Chemotherapy Hydroxyurea treatment can control and maintain the white cell count in the chronic phase but usually needs to be given indefinitely. A typical regimen would be to start with 1.0–2.0 g/day and then to reduce this in weekly increments to a maintenance dosage of 0.5–1.5 g/day. The alkylating agent busulfan is also effective in controlling the disease but has considerable long-term side-effects and is now reserved for patients who are intolerant of hydroxyurea. Imatinib has now largely replaced both drugs.

α-Interferon This was often used after the white cell count had been controlled by hydroxyurea but has now been superceded by imatinib. A typical regimen would be from 3 (to 9) megaunits between three to seven times each week given as a subcutaneous injection. The aim is to keep the white cell count low (around 4×10^9/L). Almost all patients have symptoms of a 'flu-like' illness in the first few days of treatment which responds to paracetamol and gradually wears off. More serious complications include anorexia, depression and cytopenias (see Table 11.1). A minority (approximately 15%) of patients may achieve long-term remission with loss of the Ph chromosome on cytogenetic analysis although the *BCR-ABL* fusion gene can still be detected by PCR. Interferon produces an overall prolongation of the chronic phase with increased life expectancy. Combinations of interferon with pulses of cytosine arabinoside may be more effective than interferon alone.

Stem cell transplantation Allogeneic stem cell transplantation (SCT) is the only established curative treatment for CML but, because of the risk, is usually reserved for imatinib failures. The results are better when it is performed in chronic rather than acute or accelerated phases. Only patients below approximately 65 years of age can tolerate SCT (low intensity in the older group) and only 30% of these will have a matched sibling. The 5-year survival is approximately 50–70% although this is reduced by approximately 10% if transplantation is delayed for

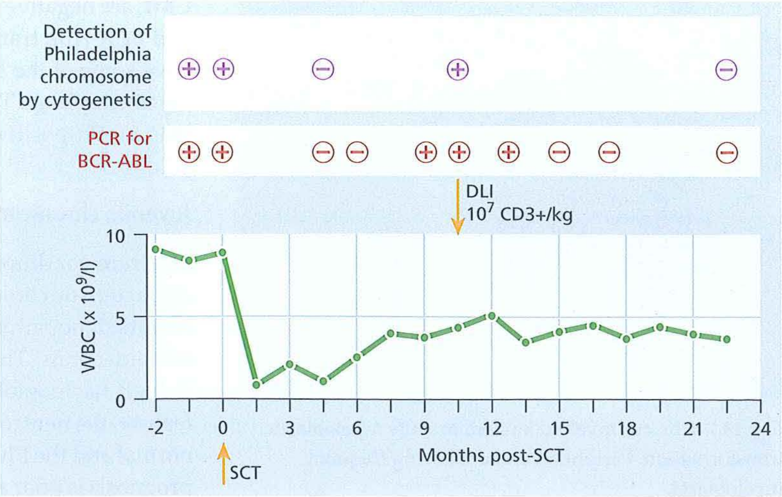


Fig. 13.6 Example of donor leucocyte infusion (DLI) in the treatment of chronic myeloid leukaemia (CML) which relapsed following allogeneic stem cell transplantation (SCT). Polymerase chain reaction (PCR) analysis of the blood for the *BCR-ABL* transcript shows that there was transient loss of the transcript but molecular and cytogenetic relapse occurred at 10 months. One infusion of donor leucocytes led to re-establishment of a durable complete remission.

more than 1 year following diagnosis. Although international bone marrow donor panels are playing an increasingly important part in providing human leucocyte antigen (HLA) matching unrelated donors, allogeneic SCT can only be offered to a minority of patients. Leukaemia relapse post-transplant is a significant problem but donor leucocyte infusions are highly effective in CML (Fig. 13.6 and p. 261), particularly if relapse is diagnosed early by molecular detection of the *BCR-ABL* transcript.

Course and prognosis

CML usually shows an excellent response to imatinib in the chronic phase. Survival is now much prolonged by imatinib. It is possible but not yet established that the best responders to imatinib may never relapse. If death occurs it is usually from terminal acute transformation or from intercurrent haemorrhage or infection. The patients may be divided into prognostic groups according to age, spleen size, platelet count, peripheral blood or bone marrow blast cells on presentation. These are only rough guides to outcome. Ease of response to imatinib is most important.

Accelerated phase disease and blastic transformation

Acute transformation (20% or more blasts in the marrow) may occur rapidly over days or weeks (Fig. 13.7). More commonly, the patient has an accelerated phase with anaemia, thrombocytopenia and an increase in basophils, eosinophils or blast cells in the blood and marrow. The spleen may be enlarged despite control of the blood count and the marrow may become fibrotic. The patient may be in this phase for several months during which the disease is less easy to control than in the chronic phase. In both the accelerated and acute phases, new chromosome abnormalities are often present. In approximately one-fifth of cases, acute transformation is lymphoblastic and patients may be treated in a similar way to ALL with a number of patients returning to the chronic phase for months or even a year or two. In the majority, transformation is into acute myeloid leukaemia (AML) or mixed types. These are more difficult to treat and survival is rare beyond a few months. Imatinib is valuable in the management of blastic transformation but resistance to treatment usually occurs within a few weeks. New tyrosine kinase inhibitors aimed at overcoming resistance to imatinib are in dasatinib and nilotinib (p. 178) trials. SCT is usually offered to younger patients with an HLA matching donor but the results are less good than in chronic phase.

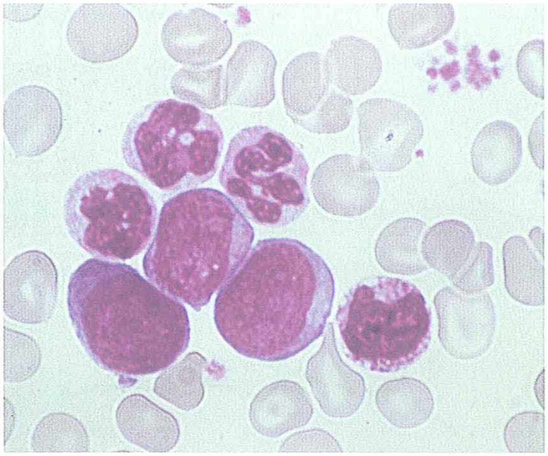


Fig. 13.7 Chronic myeloid leukemia: acute myeloblastic transformation. Peripheral blood showing frequent myeloblasts.

Variants of chronic myeloid leukaemia

Philadelphia-negative chronic myeloid leukaemia

Less than 5% of patients with features suggestive of CML are negative for the Philadelphia chromosome and *BCR-ABL* translocation. These patients usually have some of the haematological features typical of myelodysplasia. The prognosis appears to be worse than for Ph-positive CML.

Juvenile chronic myeloid leukaemia

This rare condition affects young children and has characteristic clinical features including skin rashes, lymphadenopathy, hepatosplenomegaly and recurrent infections. The blood film shows monocytosis. A high haemoglobin F level is a useful diagnostic feature, the neutrophil alkaline phosphatase score is normal and the Ph chromosome test is negative. The prognosis is poor and SCT is the treatment of choice.

Eosinophilic leukaemia and chronic neutrophilic leukaemia

These are rare conditions in which there is a relatively pure proliferation of mature cells. Some patients with idiopathic hypereosinophilia have an interstital deletion of chromosome 4 that results in a *FIP1L1-PDGFRA* fusion gene. These patients are often responsive to low doses of imatinib (p. 104). Patients with chronic neutrophilic leukaemia may have mild splenomegaly and in general the prognosis is good.

Chronic myelomonocytic leukaemia

This is classified with the myelodysplastic syndromes and with the myeloproliferative disorders (p. 186).

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CHAPTER 14

14 Myelodysplasia

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Myelodysplasia (myelodysplastic syndromes)

This is a group of clonal disorders of multipotent haemopoietic stem cells which are characterized by increasing bone marrow failure with quantitative and qualitative abnormalities of all three myeloid cell lines. A hallmark of the disease is ineffective haemopoiesis so that cytopenias often accompany a marrow of normal or increased cellularity. There is a tendency to progress to acute myeloid leukaemia (AML), although death often occurs before this develops. In most cases, the disease is *primary* but in a significant proportion of patients the disease is *secondary* to chemotherapy and/or radiotherapy which has been given for treatment of another malignancy.

Pathogenesis

The pathogenesis of myelodysplastic syndromes (MDS) is unclear but is presumed to start following genetic damage to a multipotent haemopoietic progenitor cell. This leads to increased stem cell proliferation but ineffective differentiation and maturation, leading to the paradox of a hypercellular bone marrow but a pancytopenia in peripheral blood. A high rate of apoptosis is present in bone marrow precursors. The immune system may have a role in suppressing bone marrow function; immunosuppression is sometimes used in treatment (see below).

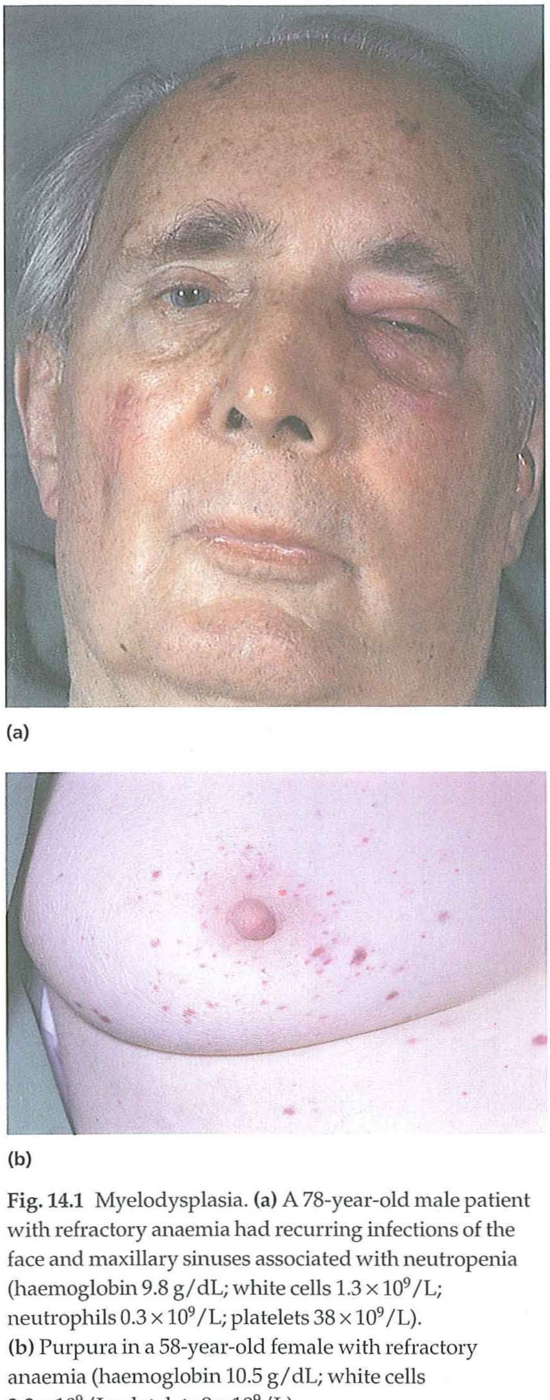
Clinical features

The disease has an incidence of 4 in 100 000 and a slight male predominance. Over half of patients are over 70 years and fewer than 25% are less than 50 years old. The evolution is often slow and the disease may be found by chance when a patient has a blood count for some unrelated reason. The symptoms, if present, are those of anaemia, infections or of easy bruising or bleeding (Fig. 14.1). In some patients transfusion-dependent anaemia dominates the course, while in others recurring infections or spontaneous bruising and bleeding are the major clinical problems. Because the neutrophils, monocytes and platelets are often functionally impaired, spontaneous infections in some cases, or bruising or bleeding in others, may occur out of proportion to the severity of the cytopenia. The spleen is not usually enlarged.

Dysplastic features in bone marrow may be seen in a wide range of conditions such as excess alcohol intake, megaloblastic anaemia, recovery from cytotoxic chemotherapy and response to growth-factor therapy and these must be ruled out before making a diagnosis of MDS.

Laboratory findings

Peripheral blood Pancytopenia is a frequent finding. The red cells are usually macrocytic or dimorphic but occasionally hypochromic; normoblasts may be present. The reticulocyte count is low. Granulocytes



(a)

(b)

Fig. 14.1 Myelodysplasia. **(a)** A 78-year-old male patient with refractory anaemia had recurring infections of the face and maxillary sinuses associated with neutropenia (haemoglobin 9.8 g/dL; white cells 1.3×10^9/L; neutrophils 0.3×10^9/L; platelets 38×10^9/L). **(b)** Purpura in a 58-year-old female with refractory anaemia (haemoglobin 10.5 g/dL; white cells 2.3×10^9/L; platelets 8×10^9/L).

are often reduced in number and frequently show lack of granulation (Fig. 14.2). Their chemotactic, phagocytic and adhesive functions are impaired. The Pelger abnormality (single or bilobed nucleus) is often present. The platelets may be unduly large or small and are usually decreased in number but in 10% of cases are elevated. In poor prognosis cases variable numbers of myeloblasts are present in the blood.

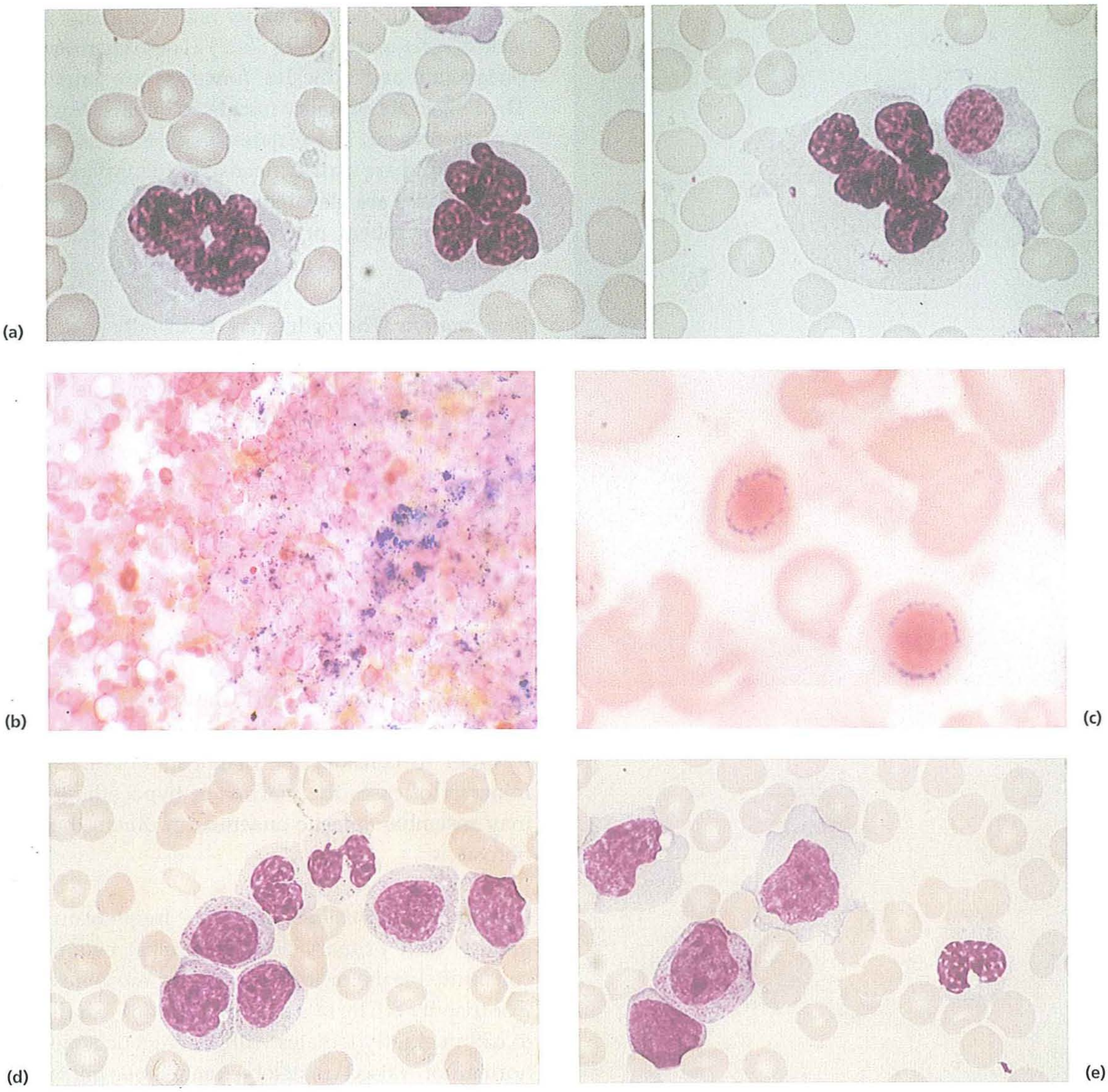
Bone marrow The cellularity is usually increased but is decreased in approximately 20% of cases. Multinucleate normoblasts and other dyserythropoietic features are seen (Fig. 14.2). The appearance of ring sideroblasts is caused by iron deposition in the mitochondria of erythroblasts. The granulocyte precursors show defective primary and secondary granulation; cells that are difficult to identify as either agranular myelocytes, monocytes or promonocytes are frequent. Megakaryocytes are abnormal with micronuclear, small binuclear or polynuclear forms. A small number of dysplastic cells may be seen in marrow from normal elderly individuals so at least 10% of the cells in a lineage should be dysplastic in order to consider the diagnosis of MDS. In a minority of cases the marrow is hypocellular and may resemble aplastic anaemia; in others there is fibrosis.

Cytogenetic abnormalities are more frequent in secondary than primary MDS and most commonly constitute partial or total loss of chromosomes 5 or 7 or trisomy 8. The loss of chromosome 5 bands q13 to q33 in elderly females with macrocytic anaemia, normal or raised platelet counts and micromegakaryocytes has been termed the 5q-syndrome and has a good prognosis. *RAS* oncogene (usually *N-RAS*) mutations occur in about 20% of cases and mutations of *FMS* in approximately 15%.

Classification of myelodysplastic syndromes

The myelodysplastic syndromes are classified on the basis of the blood count, their morphological appearance and the number of blast cells in blood or bone marrow (Table 14.1).

Although the classification appears rather complex, the principles are:



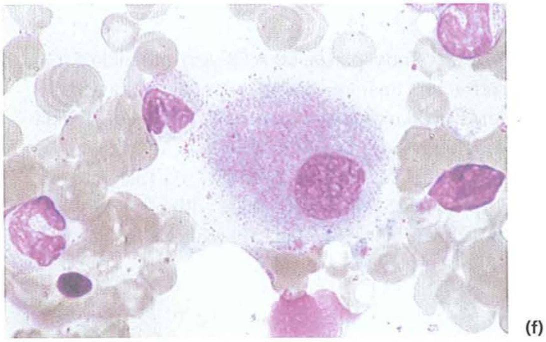


Fig. 14.2 Myelodysplasia: appearances of the peripheral blood and bone marrow. **(a)** Multinucleate polychromatic erythroblasts. **(b)** Perls' stain showing iron overload in macrophages of a bone marrow fragment. **(c)** Ring sideroblasts. **(d)** White cells showing pseudo-Pelger cells, agranular myelocytes and neutrophils. **(e)** Monocytoid cells and an agranular neutrophil. **(f)** Mononuclear megakaryocyte.

Table 14.1 The World Health Organization (WHO) classification of myelodysplasia.

Subtype	Blood	Bone marrow
Refractory anaemia (RA)	Anaemia, no blasts	Erythroid dysplasia only, <5% blasts
Refractory anaemia with ringed sideroblasts (RARS)	Anaemia, no blasts	Erythroid dysplasia only and >15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Bi- or pancytopenia or more cell lineages	Dysplasia in >10% of cells in ≥2 myeloid lineages
Refractory cytopenia with multilineage dysplasia (RCMD) and ringed sideroblasts	Bi- or pancytopenia or more cell lineages and	Dysplasia in >10% of cells in ≥2 myeloid lineages >15% ringed sideroblasts
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenias; <5% blasts no Auer rods	Uni- or multilineage dysplasia 5–9% blasts
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenias or 5–19% blasts or Auer rods	Uni- or multilineage dysplasia 10–19% blasts or Auer rods
Myelodysplasia syndrome unclassified (MDS-U)	Cytopenias no blasts or Auer rods	Myeloid or megakaryocytic dysplasia <5% blasts
MDS associated with isolated del (5q)	Anaemia; normal or increased platelets	Megakaryocytes with hypolobated nuclei <5% blasts

Patients with dysplastic features and over 20% blast cells in the bone marrow are considered to have *acute myeloid leukaemia with multilineage dysplasia* (p. 366).

- Dysplasia may be present solely in red cells (*refractory anaemia*) or present in two or more myeloid lineages (*refractory cytopenia with multilineage dysplasia*, RCMD).
- Both of these subsets can also be associated with *ring sideroblasts* and these subtypes are classified separately.
- If the blast cell count is increased in blood or bone marrow, the diagnosis is made of *refractory anaemia with excess blasts* and these subtypes have a poor prognosis.
- *5q-Syndrome* receives its own classification. It has a particularly good prognosis (Table 14.2).
- Cases with unilineage dysplasia of myeloid or megakaryocytic lineage are termed *unclassified*.

Treatment

This is often extremely difficult because chemotherapy rarely cures the disease and intensive or even low-dose chemotherapy may in some cases make the situation worse rather than better.

Table 14.2 World Health Organization (WHO) classification: clinical and laboratory features.

Subtype	MDS (%)	Cytogenetic abnormalities (%)	Median survival (months)
RA	5–10	25	66
RARS	10	<10	72
RCMD	24	50	33
RCMD-RS	15	50	33
RAEB	40	30–50	10 (RAEB-1)
Isolated del 5q	5	100	116

MDS, myelodysplastic syndromes; RA, refractory anaemia; RAEB, RA with excess blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts.

Low-risk myelodysplastic syndromes

Patients with less than 5% blasts in the marrow and only one cytopenia and favourable cytogenetics are defined as having low-grade MDS. They are usually

managed conservatively with red cell transfusions, platelet transfusions or antibiotics as required. Attempts may be made to improve marrow function with haemopoietic growth factors, either singly or in combination. Erythropoietin (Epo) benefits the anaemia in many cases of refractory anaemia and may obviate the need for transfusions. Granulocyte colony-stimulating factor (G-CSF) shows synergy with Epo and may increase the response rate. Ciclosporin or antilymphocyte globulin (ALG) occasionally improve patients, particularly those with a hypocellular bone marrow. In the long term, iron overload may be a problem after multiple transfusions; iron chelation therapy (p. 83) should be started after 30–50 units have been transfused and if the anaemia and the need for transfusion continues to be the dominant problem. Other agents (e.g. thalidomide or its derivative Lenalidomide) may be of benefit, especially in the 5q-syndrome. In selected patients, standard or low-intensity allogeneic transplantation offers a permanent cure.

High-risk myelodysplastic syndromes

In these patients, with 5% or more blasts in the marrow and often unfavourable cytogenetics and pancytopenia, a variety of treatments have been attempted to improve the overall prognosis with varying degrees of success. These treatments extend from general support alone to intensive chemotherapy.

General support care only This is most suitable in elderly patients with other major medical problems. Transfusions of red cells and platelets, and therapy with antibiotics and antifungals, are given as needed. Fewer patients in the high-risk group with anaemia are helped by recombinant Epo, even with the addition of G-CSF, than in the low-risk group.

Single-agent chemotherapy Hydroxyurea, etoposide, mercaptopurine or low-dose cytosine arabinoside may be given with some benefit to patients with refractory anaemia with excess blasts (RAEB). The demethylating agents 5′ azacytidine or decitabine have been of benefit to approximately 60% of patient trials.

Intensive chemotherapy Chemotherapy as given in AML (p. 169) may be tried in high-risk patients. Although the majority of patients may expect to obtain a remission, relapse is almost inevitable and frequently occurs within a few months. The risks of intensive chemotherapy are great because prolonged pancytopenia may occur in some cases without normal haemopoietic regeneration, presumably because normal stem cells are not present.

Stem cell transplantation SCT offers the prospect of complete cure for MDS and the advent of non-myeloablative conditioning is increasing the age range of patients that may be treated. SCT is usually carried out in MDS without a complete remission being first obtained with chemotherapy, although in high-risk cases initial chemotherapy may be tried to reduce the blast proportion and the risk of recurrence of the MDS. Older age, poor-risk cytogenetics, advanced disease and treatment-related MDS all increase the risk of an unfavourable outcome.

Myelodysplastic/myeloproliferative diseases

These disorders are characterized by the presence of dysplastic features but also increased number of circulating cells in one or more lineages (Table 14.3).

Chronic myelomonocytic leukaemia

This constituted approximately 20% of cases of

Table 14.3 The World Health Organization (WHO) classification of myelodysplastic/myeloproliferative diseases.

Chronic myelomonocytic leukaemia	CMML
Atypical chronic myeloid leukaemia*	aCML
Juvenile myelomonocytic leukaemia	JMML
Myelodysplastic/myeloproliferative disease	Unclassifiable

* This is discussed in Chapter 13.

MDS when it was classified with them. It is defined by a persistent monocytosis of $>1.0 \times 10^9/L$. The total white cell count is usually raised and may exceed $100 \times 10^9/L$. Patients may develop skin rashes and around half have splenomegaly. Gum hypertrophy and lymphadenopathy may also be present. Treatment is difficult although hydroxyurea, etoposide or mercaptopurine may be useful. SCT may be tried in younger patients. Median survival is approximately 2 years, with increased marrow blasts a predictor of poor outcome.

Juvenile myelomonocytic leukaemia

This presents in the first 4 years of life and has features of both myelodysplasia and a myeloproliferative disease. There is often a skin rash, hepatosplenomegaly and lymphadenopathy. There is a monocytosis to $>1.0 \times 10^9/L$, clonal cytogenetic changes but not the *BCR-ABL* fusion gene. The only curative treatment is allogeneic SCT. If untreated, death usually occurs within 4 years, often from acute transformation with leukaemic infiltration (e.g. of the lungs).

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CHAPTER 15

15 The chronic lymphoid leukaemias

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Several disorders are included in this group characterized by accumulation in the blood of mature lymphocytes of either B- or T-cell type (Table 15.1). There is considerable overlap with the lymphomas. In many cases of non-Hodgkin's lymphoma, lymphoma cells are found in the blood and the distinction between chronic leukaemia and lymphoma is arbitrary, depending on the relative proportion of the disease in soft tissue masses compared to blood and bone marrow. In general, the diseases are incurable but tend to run a chronic and fluctuating course.

Table 15.1 Classification of the chronic lymphoid leukaemias and leukaemia/lymphoma syndromes.*

B-cell	T-cell
Chronic lymphoid leukaemias	
Chronic lymphocytic leukaemia (CLL)	Large granular lymphocytic leukaemia
Prolymphocytic leukaemia (PLL)	T-cell prolymphocytic leukaemia (T-PLL)
Hairy cell leukaemia (HCL)	
Plasma cell leukaemia	
Leukaemia/lymphoma syndromes	
Splenic lymphoma with villous lymphocytes	Sézary syndrome
Follicular lymphoma	Adult T-cell leukaemia/lymphoma
Mantle cell lymphoma	Large cell lymphoma
Lymphoplasmacytic lymphoma	
Large cell lymphoma	

* WHO classification see page 367.

Diagnosis

This group is characterized by a chronic persistent lymphocytosis. Subtypes are distinguished by morphology, immunophenotype and cytogenetics. DNA analysis may be useful in showing a monoclonal rearrangement of either immunoglobulin or T-cell receptor genes.

B-cell diseases

Chronic lymphocytic leukaemia

Chronic lymphocytic leukaemia (CLL) is by far the most common of the chronic lymphoid leukaemias and has a peak incidence between 60 and 80 years of age. The aetiology is unknown but there are geographical variations in incidence. It is the most common of the leukaemias in the Western world but rare in the Far East. In contrast to other forms of leukaemia there is no higher incidence after previous chemotherapy or radiotherapy. There is a sevenfold increased risk of CLL in the close relatives of patients. The tumour cell appears to be a relatively mature B cell with weak surface expression of immunoglobulin (Ig) M or IgD. The cells accumulate in the blood, bone marrow, liver, spleen and lymph nodes as a result of a prolonged lifespan with impaired apoptosis.

Clinical features

1 The disease occurs in older subjects with only 15%

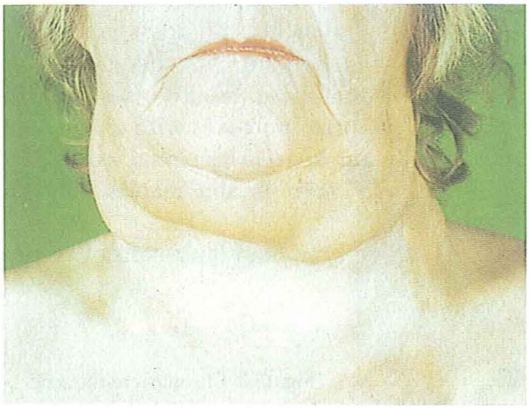


Fig. 15.1 Chronic lymphocytic leukaemia: bilateral cervical lymphadenopathy in a 67-year-old woman. Haemoglobin 12.5 g/dL; white blood count 150×10^9/L (lymphocytes 146×10^9/L); platelets 120×10^9/L.

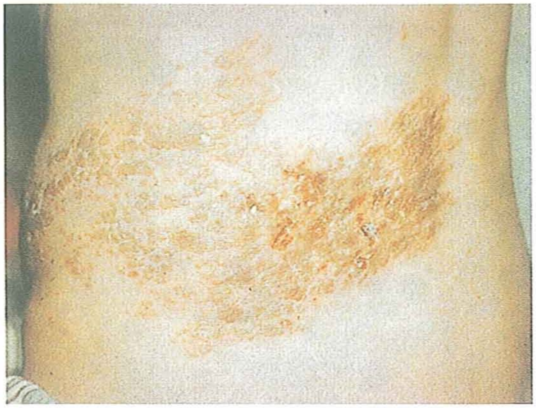


Fig. 15.2 Chronic lymphocytic leukaemia: herpes zoster infection in a 68-year-old female.

of cases before 50 years of age. The male to female ratio is 2 : 1.

2 Most cases are diagnosed when a routine blood test is performed. With increasing routine medical check-ups, this proportion is rising.

3 Symmetrical enlargement of cervical, axillary or inguinal lymph nodes is the most frequent clinical sign (Fig. 15.1). The nodes are usually discrete and non-tender. Tonsillar enlargement may be a feature.

4 Features of anaemia may be present. Patients with thrombocytopenia may show bruising or purpura.

5 Splenomegaly and, less commonly, hepatomegaly are common in later stages.

6 Immunosuppression is a significant problem resulting from hypogammaglobulinaemia and cellular immune dysfunction. Early in the disease course bacterial infections predominate but with advanced disease viral and fungal infections such as herpes zoster are also seen (Fig. 15.2).

Laboratory findings

1 Lymphocytosis. The absolute lymphocyte count is $>5 \times 10^9$/L and may be up to 300×10^9/L or more. Between 70 and 99% of white cells in the blood film appear as small lymphocytes. Smudge or smear cells are also present (Fig. 15.3).

2 Immunophenotyping of the lymphocytes shows them to be B cells (surface $CD19^+$), weakly expressing surface immunoglobulin (IgM or IgD). This is shown to be monoclonal because of expression of only one form of light chain (κ or λ; p. 141). Characteristically, the cells are also surface $CD5^+$ and $CD23^+$ but $CD79b^-$ and $FMC7^-$ (Table 15.2). Similar clones are found in some healthy subjects but with a normal total lymphocyte count.

3 Normocytic normochromic anaemia is present in later stages as a result of marrow infiltration or hypersplenism. Autoimmune haemolysis may also occur (see below). Thrombocytopenia occurs in many patients.

4 Bone marrow aspiration shows lymphocytic replacement of normal marrow elements. Lymphocytes comprise 25–95% of all the cells. Trephine biopsy reveals nodular, diffuse or interstitial involvement by lymphocytes.

5 Reduced concentrations of serum immunoglobulins are found and this becomes more marked with advanced disease. Rarely a paraprotein is present.

6 Autoimmunity directed against cells of the haemopoietic system is common. Autoimmune haemolytic anaemia is most frequent but immune thrombocytopenia (p. 283), neutropenia and red cell aplasia are also seen.

Prognostic markers

Cytogenetics The four most common chromosome abnormalities are deletion of 13q14, trisomy 12, deletions at 11q23 and structural abnormalities of

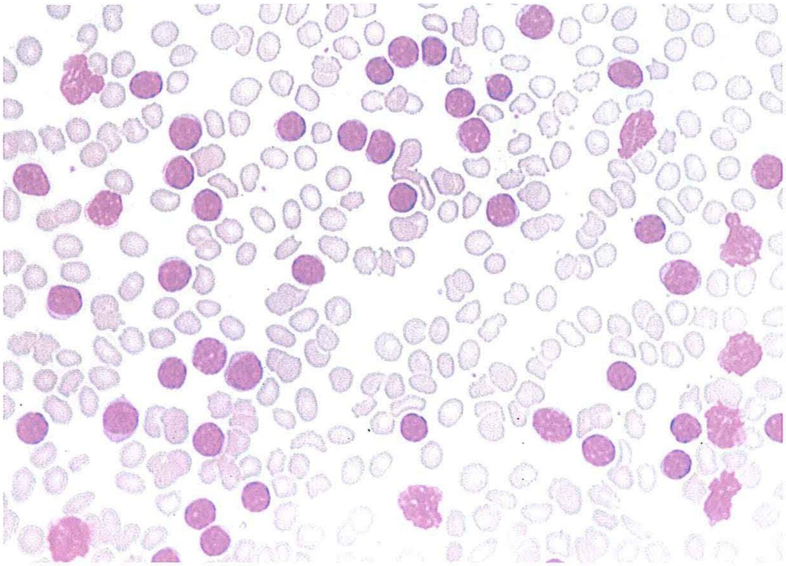


Fig. 15.3 Chronic lymphocytic leukaemia: peripheral blood film showing lymphocytes with thin rims of cytoplasm, coarse condensed nuclear chromatin and rare nucleoli. Typical smudge cells are present.

Table 15.2 Immunophenotype of the chronic B-cell leukaemias/lymphomas (all cases $CD19^+$).

	CLL	PLL	HCL	FL	MCL
SIg	Weak	++	++	++	+
CD5	+	–	–	–	+
CD22/FMC7	–	+	+	+	+
CD23	+	–	–	–	–
CD79b	–	++	–/+	++	++
CD103*	–	–	+	–	–

CLL, chronic lymphocytic leukaemia; FL, follicular lymphoma; HCL, hairy cell leukaemia; MCL, mantle cell lymphoma; PLL, prolymphocytic leukaemia.
* CD103 is positive only in HCL.

17p involving the p53 gene. These abnormalities carry prognostic significance (Table 15.3). The 13q14 deletion prevents expression of microRNAs which control expression of proteins relevant to the CLL lifespan (p. 137). Expression of a novel gene CLLU.1 is substantially greater in unmutated than mutated cases.

Somatic hypermutation of the immunoglobulin genes When B cells recognize antigen in the germinal centre of secondary lymphoid tissues they undergo a process called *somatic hypermutation* in which random mutations occur in the immunoglobulin heavy-chain gene (p. 113). In CLL, the *IGVH* gene shows evidence of this hypermutation in approximately 50% of cases whereas in the other cases the *VH* genes are unmutated. CLL with unmutated immunoglobulin genes has an unfavourable prognosis (Table 15.3).

Tumour cell phenotype ZAP-70 is a protein tyrosine kinase that is involved in cell signalling following recognition of antigen by antigen receptors on lymphocytes. Its expression is normally restricted to T cells but is also aberrantly expressed in cases of CLL. Its expression is associated with an unfavourable clinical outcome.

Staging

It is useful to stage patients at presentation both for prognosis and for deciding on therapy. The Rai and Binet staging systems are shown in Table 15.4. Typical survival ranges from 12 years for Rai stage 0 to less than 3 years for stage IV.

Treatment

Cures are rare in CLL and so the approach to therapy is conservative, aiming for symptom control rather than a normal blood count. Indeed, chemotherapy given too early in the disease can

Table 15.3 Prognostic factors in chronic lymphocytic leukaemia.

	Good	Bad
Stage	Binet A (Rai 0–I)	Binet B, C (Rai II–IV)
Sex	Female	Male
Lymphocyte doubling time	Slow	Rapid
Bone marrow biopsy appearance	Nodular	Diffuse
Chromosomes	Deletion 13q14	Trisomy 12; deletion 17p; deletion 11q23
VH immunoglobulin genes	Hypermutated	Unmutated
ZAP expression	Low	High
CD38 expression	Negative	Positive
CLLU.1 expression	Low	High
LDH	Normal	Raised

LDH, lactate dehydrogenase.

Table 15.4 Staging of chronic lymphocytic leukaemia (CLL).
(a) Rai classification

Stage	
0	Absolute lymphocytosis $>15 \times 10^9/L$*
I	As stage 0 + enlarged lymph nodes (adenopathy)
II	As stage 0 + enlarged liver and/or spleen ± adenopathy
III	As stage 0 + anaemia (Hb <10.0 g/dL)* ± adenopathy ± organomegaly
IV	As stage 0 + thrombocytopenia (platelets $<100 \times 10^9/L$)* ± adenopathy ± organomegaly

(b) International Working Party classification (From J.L. Binet *et al.* 1981)

Stage	Organ enlargement*	Haemoglobin† (g/dL)	Platelets† ($\times 10^9/L$)
A (50–60%)	0, 1 or 2 areas		
B (30%)	3, 4 or 5 areas	≥10	≥100
C (<20%)	Not considered	<10	and/or <100

* One area = lymph nodes >1 cm in neck, axillae, groins or spleen, or liver enlargement.
† Secondary causes of anaemia (e.g. iron deficiency) or autoimmune haemolytic anaemia or autoimmune thrombocytopenia must be treated before staging.

shorten rather than prolong life expectancy. Many patients never receive treatment. Treatment is given for troublesome organomegaly, haemolytic episodes and bone marrow suppression. The lymphocyte count alone is not a good guide to treatment. Usually, patients in Binet stage C will need treatment as will some in stage B.

Chemotherapy

Chlorambucil This oral alkylating agent can be used as a daily treatment (e.g. 4–6 mg/day) or in a monthly cycle (e.g. 10 mg/m^2/day for 7 days) and is effective in reducing disease bulk in the majority of cases. Typically the drug will need to be given for

several months after which a remission of variable duration will be obtained. Chlorambucil can be reintroduced when required although resistance may develop.

Purine analogues These drugs are effective in the treatment of chronic lymphoid leukaemias and lymphomas. The most effective agent in CLL appears to be fludarabine. Its place in the overall management of CLL is currently under trial. It is the drug of first choice in younger patients as well as having value in patients resistant to chlorambucil. Myelosuppression and prolonged reduction of CD4 (helper) T lymphocytes lead to an increased infection risk and prophylaxis against *Pneumocystis carinii* infection with co-trimoxazole is given until CD4 counts recover. The combination of fludarabine with cyclophosphamide (FC) is more effective than fludarabine alone and fludarabine, cyclophosphamide and rituximab (FCR) increases further the response rate and duration of response.

Monoclonal antibodies Campath-1H (anti-CD52) is a monoclonal antibody that is effective in killing white cells by complement fixation. It is given intravenously or subcutaneously over several weeks and as well as being valuable in resistant and relapsed disease, it may also find a role earlier in treatment. It is highly immunosuppressive and antibacterial and antiviral prophylaxis is advisable.

CD20 expression on CLL cells is not high; rituximab (p. 217), however, appears to be valuable in combination therapy (e.g. with fludarabine). It also has a role in treating autoimmune haemolytic anaemia, thrombocytopenia and red cell aplasia.

Corticosteroids Patients in bone marrow failure should be treated initially with prednisolone alone until there is significant recovery of the platelet, neutrophil and haemoglobin levels. The peripheral lymphocyte count initially rises as infiltrated organs shrink, but later the count falls. Corticosteroids are also indicated in autoimmune haemolytic anaemia or thrombocytopenia.

Other forms of treatment

Radiotherapy This is valuable in reducing the size of bulky lymph node groups that are unresponsive to chemotherapy. Radiotherapy to the spleen may be valuable in late-stage disease.

Combination chemotherapy Cyclophosphamide, hydroxodaunorubicin, oncovin (vincristine) and prednisolone (CHOP; p. 213) with rituximab is sometimes used in late-stage cases and in patients refractory to chlorambucil.

Ciclosporin Red cell aplasia may respond to ciclosporin.

Splenectomy This is generally reserved for those patients with immune-mediated cytopenias that do not respond to short courses of steroids or those with painful bulky enlargement of the spleen.

Immunoglobulin replacement Immunoglobulin (e.g. 400 mg/kg/month by intravenous infusion) is useful for patients with hypogammaglobulinaemia and recurrent infections.

Stem cell transplantation This is currently an experimental approach in younger patients. Allogeneic stem cell transplantation (SCT) may be curative but has a high mortality rate. Autologous SCT after prior therapy with fludarabine and other drugs is undergoing clinical trials.

Course of disease

Many patients in Binet stage A or Rai stage 0 or I never need therapy and this is particularly likely for those with favourable prognostic markers (Table 15.3). For those who do need treatment a typical pattern is that of a disease that is responsive to several courses of chemotherapy before the gradual onset of extensive bone marrow infiltration, bulky disease and recurrent infection. The disease may transform into a localized high-grade lymphoma (Richter's transformation) or there may be the appearance of an increasing number of prolymphocytes that are resistant to treatment.

Prolymphocytic leukaemia

Although prolymphocytic leukaemia (PLL) may initially appear similar to CLL, the diagnosis is

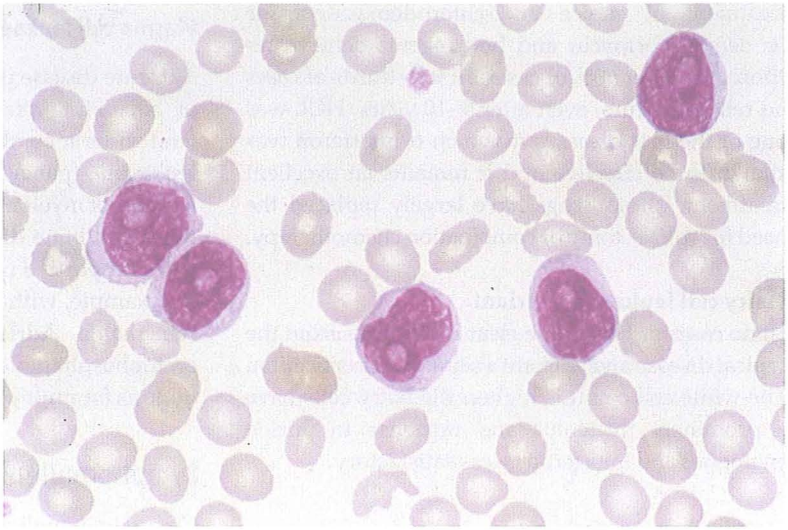


Fig. 15.4 Prolymphocytic leukaemia: blood film showing prolymphocytes that have prominent central nucleoli and an abundance of pale cytoplasm.

made by the appearance of a majority of prolymphocytes in the blood. The prolymphocyte is around twice the size of a CLL lymphocyte and has a large central nucleolus (Fig. 15.4). B-cell PLL is three times more common than T-cell PLL.

PLL and CLL also differ in their clinical features. PLL typically presents with splenomegaly without lymphadenopathy and with a high and rapidly rising lymphocyte count. Anaemia is a poor prognostic feature. Treatment is difficult in PLL. Splenectomy is usually of benefit and purine nucleoside analogues and rituximab may help.

Hairy cell leukaemia

Hairy cell leukaemia (HCL) is an uncommon B-cell lymphoproliferative disease with a male : female ratio of 4 : 1 and a peak incidence at 40–60 years. Patients typically present with infections, anaemia or splenomegaly. Lymphadenopathy is very uncommon. Pancytopenia is usual at presentation and the lymphocyte count is rarely over $20 \times 10^9/L$. Monocytopenia is a distinctive feature. The blood film reveals a variable number of unusual large lymphocytes with villous cytoplasmic projections (Fig. 15.5). Immunophenotyping shows CD22, FMC7 and CD103 positivity in most cases (Table 15.2). The hairy cells stain for tartrate-resistant acid phosphatase (TRAP). The bone marrow trephine shows a characteristic appearance of mild fibrosis and a diffuse cellular infiltrate.

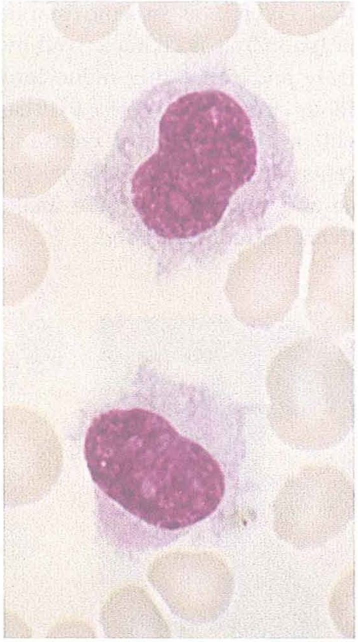


Fig. 15.5 Hairy cell leukaemia: peripheral blood film showing typical 'hairy' cells with oval nuclei and finely mottled pale grey/blue cytoplasm with an irregular edge.

There are several effective treatments for HCL and a patient can expect a long-term remission. The

treatment of choice is 2-chlorodeoxyadenosine or deoxycoformycin and both agents achieve responses in over 90% of cases. In two-thirds of cases no relapse occurs, even after 5–10 years. HCL was one of the first diseases in which α-interferon was shown to be effective and it remains an excellent treatment. These drugs have largely replaced the need for splenectomy or combination chemotherapy.

Hairy cell leukaemia variant

Some cases of HCL have clear differences from the typical disease and warrant a separate classification. The white cell count is higher, the hairy cells have a prominent nucleolus and response to purine analogues and interferon is less satisfactory.

Splenic lymphoma with villous lymphocytes

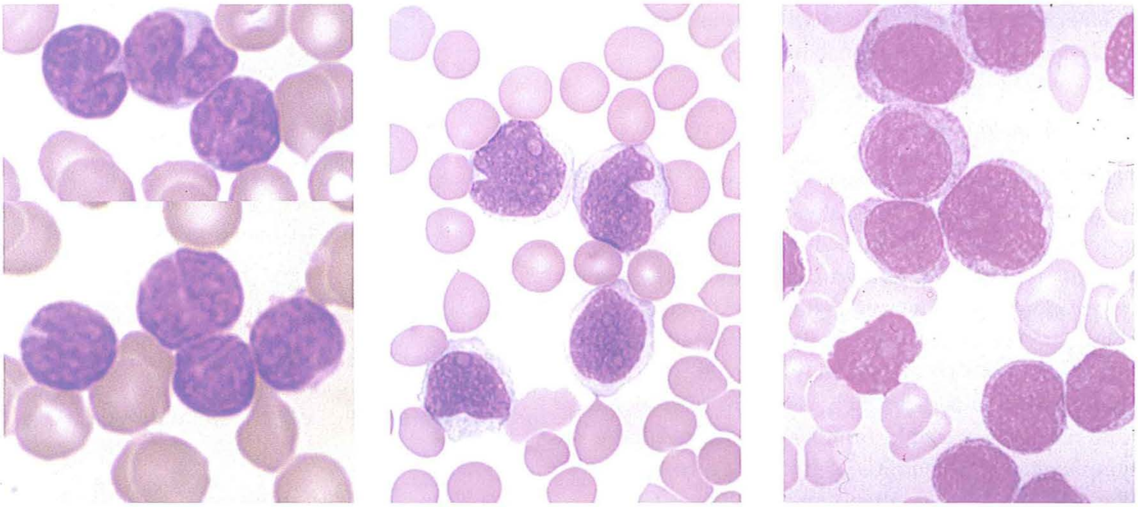
This is now classified within the World Health Organization (WHO) as splenic marginal zone lymphoma (p. 367). It is characterized by massive splenomegaly and circulating monoclonal B cells with a villous appearance. It is a disease of the elderly with a benign clinical course. Although many patients will not need treatment, splenectomy is valuable and purine nucleoside analogues are also effective. The outlook is good.

Plasma cell leukaemia

This rare disease is characterized by a high number of circulating plasma cells. The clinical features tend to be a combination of those found in acute leukaemia (pancytopenia and organomegaly) with features of myeloma (hypercalcaemia, renal involvement and bone disease; Chapter 18). Treatment is with supportive care and systemic chemotherapy; for example, with CHOP, cyclophosphamide-VAD (vincristine, Adriamycin and dexamethasone) or cyclophosphamide, dexamethasone and thalidomide as for multiple myeloma (Chapter 18).

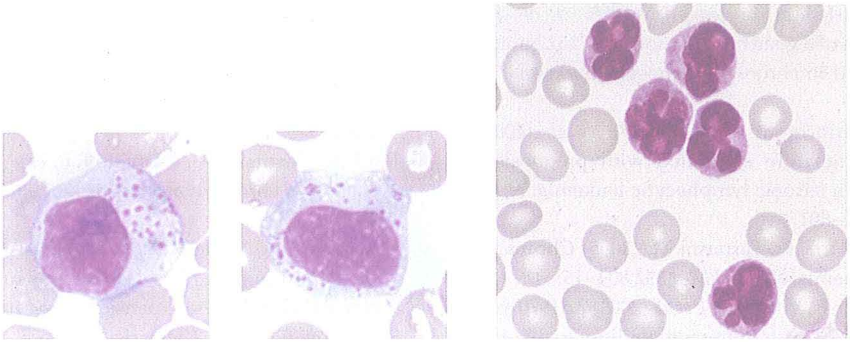
Lymphoma/leukaemia syndromes

Circulating malignant lymphoid cells occur in a variety of syndromes in association with otherwise typical cases of non-Hodgkin's lymphoma. This syndrome is most frequently seen in B-cell tumours of the follicle centre cell type (with circulating indented or cleaved nuclei; Fig. 15.6) and the course is that of the non-Hodgkin's lymphoma (p. 209). Mantle zone lymphoma is often associated with circulating B cells (Fig. 15.6) and, because these are $CD5^+$, mantle zone lymphoma may be confused with CLL. Other types of lymphoma may also show



(a) (b) (c)

Fig. 15.6 Blood involvement by malignant lymphoma: **(a)** small cleaved lymphoid cells in follicle centre cell lymphoma; **(b)** mantle cell lymphoma; **(c)** large B-cell lymphoma.



(a) (b)

Fig. 15.7 **(a)** Large granular lymphocytes in the peripheral blood. **(b)** Adult T-cell leukaemia/lymphoma. Typical convoluted lymphoid cells in peripheral blood.

tumour cells in the peripheral blood and bone marrow and in some cases it is difficult to define the disease as either lymphoma (with mainly soft tissue masses) or leukaemia.

T-cell diseases

Large granular lymphocytic leukaemia

Large granular lymphocytic leukaemia (LGL-L) is characterized by the presence of circulating lymphocytes with abundant cytoplasm and large azurophilic granules (Fig. 15.7a). Such cells may be either T or natural killer (NK) cells and show variable expression of CD16, CD56 and CD57. Cytopenia, especially neutropenia, is the main clinical problem although anaemia, splenomegaly and arthropathy with positive serology for rheumatoid arthritis are also common. The mean age is 50 years. Treatment may not be needed but, if required, steroids, cyclophosphamide, ciclosporin or methotrexate may relieve the cytopenia. Granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) have been used in cases associated with neutropenia.

Adult T-cell leukaemia/lymphoma

Adult T-cell leukaemia/lymphoma (ATLL) was the first malignancy to be associated with a human retrovirus, human T-cell leukaemia/lymphoma virus type 1 (HTLV-1). The virus is endemic in parts of Japan and the Caribbean but the disease is very rare in people who have not lived in these areas. ATLL lymphocytes have a bizarre morphology with a convoluted 'clover-leaf' nucleus and a consistent $CD4^+$ phenotype (Fig. 15.7b).

Most subjects infected with the virus do not develop the disease. The clinical presentation is often acute and dominated by hypercalcaemia, skin lesions, hepatosplenomegaly and lymphadenopathy. Diagnosis is by morphology and serology and although combination chemotherapy may be tried the prognosis is poor. Antiretroviral drugs may prove to have a valuable role.

Sézary syndrome

Patients with Sézary syndrome present with skin disease, usually a pruritic exfoliative erythroderma affecting the palms, soles and face ('red man syndrome'). Biopsy of the skin reveals lymphocytic infiltration and Sézary cells in peripheral blood have a characteristic morphology with deep nuclear clefting, similar to ATLL cells. A variety of treatments are available including chemotherapy, radiotherapy and a photoactivable drug (psoralens) combined with ultraviolet A light (PUVA).

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CHAPTER 16

16 Hodgkin's lymphoma

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Lymphomas are a group of diseases caused by malignant lymphocytes that accumulate in lymph nodes and cause the characteristic clinical features of lymphadenopathy. Occasionally, they may spill over into blood ('leukaemic phase') or infiltrate organs outside the lymphoid tissue.

The major subdivision of lymphomas is into Hodgkin's lymphoma and non-Hodgkin's lymphoma and this is based on the histological presence of Reed–Sternberg (RS) cells in Hodgkin's lymphoma.

Pathogenesis

Hodgkin's disease is a lymphoma in which RS cells are found in the disease tissue. The characteristic RS cells, and the associated abnormal mononuclear cells, are neoplastic whereas the infiltrating inflammatory cells are reactive. Immunoglobulin gene rearrangement studies suggest that the RS cell is of B-lymphoid lineage and that it is often derived from a B cell with a 'crippled' immunoglobulin gene caused by the acquisition of mutations that prevent synthesis of full-length immunoglobulin. The Epstein–Barr virus (EBV) genome has been detected in 50% or more of cases in Hodgkin tissue but its role in the pathogenesis is unclear.

Clinical features

The disease can present at any age but is rare in children and has a peak incidence in young adults. There is an almost 2 : 1 male predominance. The following symptoms are common.

1 Most patients present with painless, non-tender, asymmetrical, firm, discrete and rubbery enlargement of superficial lymph nodes (Fig. 16.1). The cervical nodes are involved in 60–70% of patients, axillary nodes in approximately 10–15% and inguinal nodes in 6–12%. In some cases the size of the nodes decreases and increases spontaneously. They may

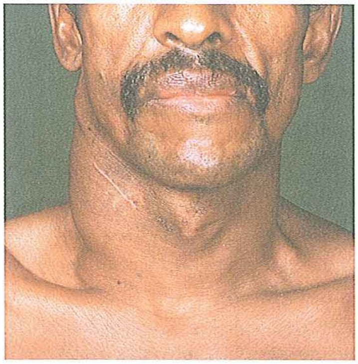


Fig. 16.1 Cervical lymphadenopathy in a patient with Hodgkin's lymphoma.

become matted. Typically the disease is localized initially to a single peripheral lymph node region and its subsequent progression is by contiguity within the lymphatic system. Retroperitoneal nodes are also often involved but usually only diagnosed by computed tomography (CT) scan.

2 Clinical splenomegaly occurs during the course of the disease in 50% of patients. The splenic enlargement is seldom massive. The liver may also be enlarged because of liver involvement.

3 Mediastinal involvement is found in 6–11% of patients at presentation. This is a feature of the nodular sclerosing type, particularly in young women. There may be associated pleural effusions or superior vena cava obstruction.

4 Cutaneous Hodgkin's disease occurs as a late complication in approximately 10% of patients. Other organs (e.g. bone marrow, gastrointestinal tract, bone, lung, spinal cord or brain) may also be involved, even at presentation, but this is unusual.

5 Constitutional symptoms are prominent in patients with widespread disease. The following may be seen:

(a) fever occurs in approximately 30% of patients and is continuous or cyclic;

(b) pruritus, which is often severe, occurs in approximately 25% of cases;

(c) alcohol-induced pain in the areas where disease is present occurs in some patients;

(d) other constitutional symptoms include weight loss, profuse sweating (especially at night), weakness, fatigue, anorexia and cachexia. Haematological and infectious complications are discussed below.

Haematological and biochemical findings

1 Normochromic, normocytic anaemia is most common. Bone marrow involvement is unusual in early disease but if it occurs bone marrow failure may develop with a leucoerythroblastic anaemia.

2 One-third of patients have a neutrophilia; eosinophilia is frequent.

3 Advanced disease is associated with lymphopenia and loss of cell-mediated immunity.

4 The platelet count is normal or increased during early disease, and reduced in later stages.

5 The erythrocyte sedimentation rate (ESR) and C-reactive protein are usually raised and are useful in monitoring disease progress.

6 Serum lactate dehydrogenase (LDH) is raised initially in 30–40% of cases.

Diagnosis and histological classification

The diagnosis is made by histological examination of an excised lymph node. The distinctive multinucleate polyploid RS cell is central to the diagnosis of the four classic types (Figs 16.2 and 16.3) and mononuclear Hodgkin cells are also part of the malignant clone. These cells stain with CD30 and CD15 but are usually negative for B-cell antigen expression. Inflammatory components consist of lymphocytes, neutrophils, eosinophils, plasma cells and variable fibrosis.

Histological classification is into five types (Table 16.1), each of which implies a different prognosis. Nodular sclerosis and mixed cellularity are most frequent. Patients with lymphocyte predominant histology have the most favourable prognosis. Nodular lymphocyte predominant does not show RS cells and has many features of non-Hodgkin's lymphoma and may be treated as such.

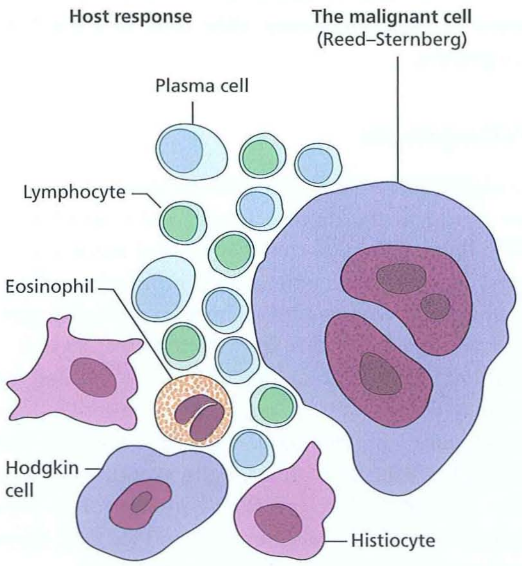
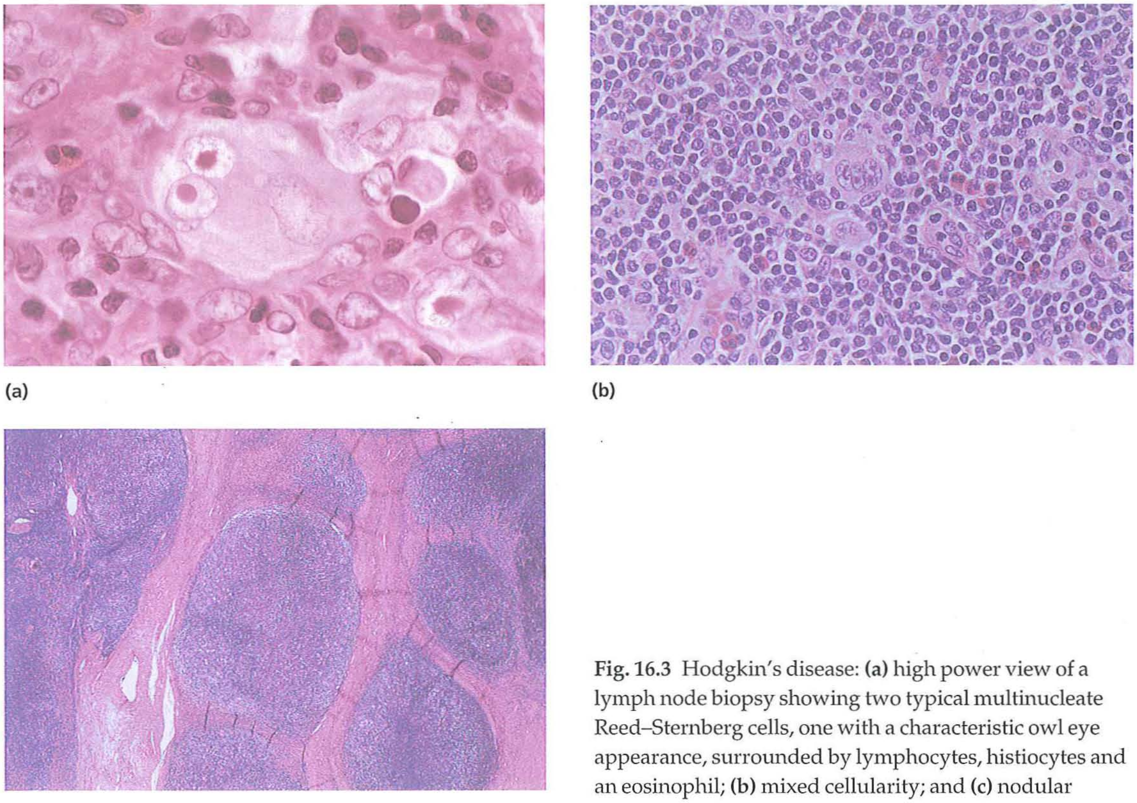


Fig. 16.2 Diagrammatic representation of the different cells seen histologically in Hodgkin's lymphoma.



(a) (b) (c)

Fig. 16.3 Hodgkin's disease: **(a)** high power view of a lymph node biopsy showing two typical multinucleate Reed–Sternberg cells, one with a characteristic owl eye appearance, surrounded by lymphocytes, histiocytes and an eosinophil; **(b)** mixed cellularity; and **(c)** nodular sclerosing Hodgkin's lymphoma.

Table 16.1 Histology of Hodgkin's lymphoma (WHO classification).

Classic Hodgkin's lymphoma	
Nodular sclerosis	Collagen bands extend from the node capsule to encircle nodules of abnormal tissue. A characteristic lacunar cell variant of the Reed–Sternberg cell is often found. The cellular infiltrate may be of the lymphocyte-predominant, mixed cellularity or lymphocyte-depleted type; eosinophilia is frequent
Mixed cellularity	The Reed–Sternberg cells are numerous and lymphocyte numbers are intermediate
Lymphocyte depleted	There is either a reticular pattern with dominance of Reed–Sternberg cells and sparse numbers of lymphocytes or a diffuse fibrosis pattern where the lymph node is replaced by disordered connective tissue containing few lymphocytes. Reed–Sternberg cells may also be infrequent in this latter subtype
Lymphocyte rich	Scanty Reed–Sternberg cells; multiple small lymphocytes with few eosinophils and plasma cells; nodular and diffuse types
Nodular lymphocyte-predominant	Reed–Sternberg cells are absent; abnormal polymorphic B cells

WHO, World Health Organization.

Clinical staging

The selection of appropriate treatment depends on accurate staging of the extent of disease (Table 16.2). Figure 16.4 shows the scheme (Ann Arbor) that is now recommended. Staging is performed by thorough clinical examination together with chest X-ray (Fig. 16.5) and CT scan to detect intrathoracic, intra-abdominal or pelvic disease (Fig. 16.6). It is also used to monitor response to therapy. Magnetic resonance imaging (MRI) scanning may be needed for particular sites (Table 16.2). Bone marrow trephine is carried out in some centres and liver biopsy may also be needed in difficult cases. Positron emission tomography (PET) scanning may also be useful in staging and PET scan combined with CT may be used in staging and to detect small foci of residual disease following treatment (Fig. 17.8).

Table 16.2 Techniques for staging of lymphoma.

Laboratory	Full blood count
	ESR
	Bone marrow aspirate and trephine (not routine)
	Liver function
	LDH
	C-reactive protein
Radiology	Chest radiograph
	CT of thorax, abdomen, chest and pelvis
Special tests	MRI
	Bone scan
	PET or PET/CT

CT, computed tomography; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PET, positron emission tomography.

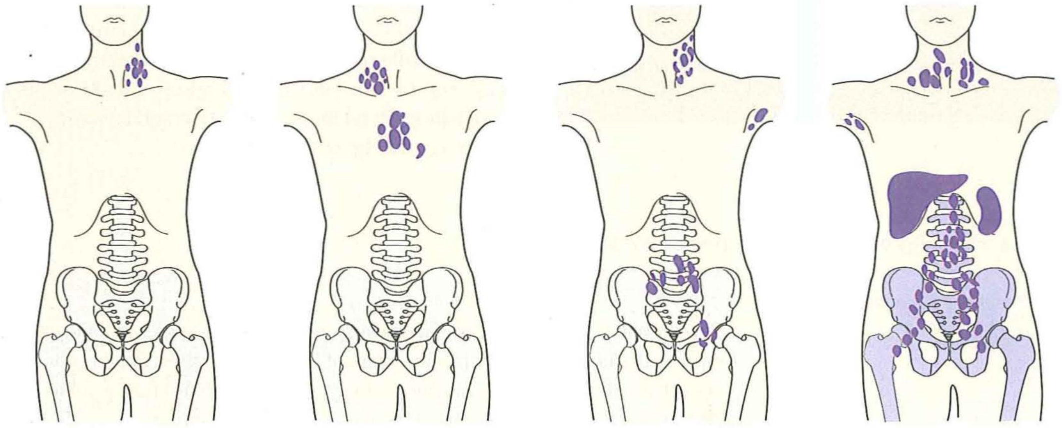


Fig. 16.4 Staging of Hodgkin's lymphoma. Stage I indicates node involvement in one lymph node area. Stage II indicates disease involving two or more lymph nodal areas confined to one side of the diaphragm. Stage III indicates disease involving lymph nodes above and below the diaphragm. Splenic disease is included in stage III but this has special significance (see below). Stage IV indicates involvement outside the lymph node areas and refers to diffuse or disseminated disease in the bone marrow, liver and other extranodal sites. NB. The stage number in all cases is followed by the letter A or B indicating the absence (A) or presence (B) of one or more of the following: unexplained fever above 38°C; night sweats; or loss of more than 10% of body weight within 6 months. Localized extranodal extension from a mass of nodes does not advance the stage but is indicated by the subscript E. Thus, mediastinal disease with contiguous spread to the lung or spinal theca would be classified as I_E. As involvement of the spleen is often a prelude to widespread haematogenous spread of the disease, patients with lymph node and splenic involvement are staged as III_S. Bulky disease (widening of the mediastinum by more than one-third, or the presence of a nodal mass >10 cm in diameter) is relevant to therapy at any stage.

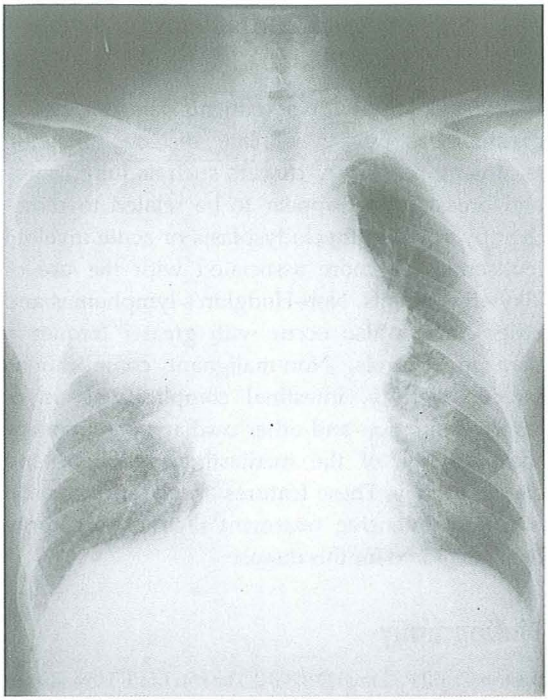


Fig. 16.5 Chest X-ray in Hodgkin's lymphoma showing widespread enlargement of hilar and mediastinal lymph nodes with associated collapse of the right upper lobe and infiltration or possibly pneumonic changes in the mid zone of the left lung.

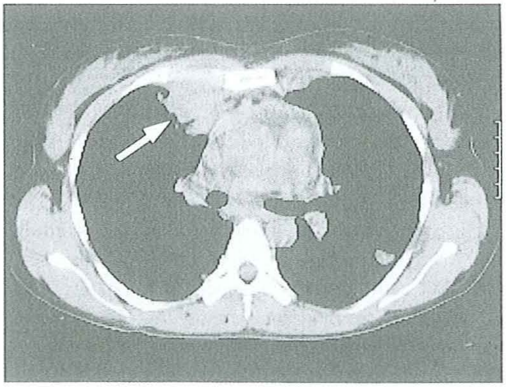


Fig. 16.6 Hodgkin's lymphoma (nodular sclerosing type): CT scan of chest showing anterior mediastinal mass of enlarged lymph nodes (arrowed).

Patients are also classified as A or B according to whether or not constitutional features (fever or weight loss) are present (Fig. 16.4).

Treatment

Treatment is with radiotherapy, chemotherapy or a combination of both. The choice depends primarily on the stage and whether clinically A or B (Fig. 16.4) although histological grading is an additional factor. Semen storage, if appropriate, should be carried out before therapy is begun.

Radiotherapy

Patients with stage I and IIA disease may be cured by radiotherapy alone. A dose of 4000 rad (40 Gy) is able to destroy lymph node Hodgkin's tissue in approximately 80% of these patients. Improved high-voltage radiotherapy techniques allow the treatment of all lymph node areas above or below the diaphragm by single 'upper mantle' or 'inverted Y' blocks. Radiotherapy also has a role in the treatment of bulky tumour masses such as mediastinal tumour that remains after chemotherapy or painful skeletal, nodal or soft tissue deposits.

Combined modality therapy

Concerns over late relapse and the long-term effects of radiotherapy have led to the development of regimens in which chemotherapy and radiotherapy are used together. This combination therapy allows short courses of chemotherapy to be combined with reduced levels of radiotherapy and the most effective combinations are being assessed in clinical trials.

Chemotherapy

Cyclical chemotherapy is used for stage III and IV disease and also in stage I and II patients who have bulky disease, type B symptoms or have relapsed following initial radiotherapy. The combination of Adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) is now most widely used. This has replaced the landmark quadruple therapy with mustine, vincristine (Oncovin), procarbazine and prednisolone (MOPP) which was introduced in the

1970s, but is more likely to cause sterility or secondary leukaemia. Variants of this such as ChlVPP (replacing mustine with chlorambucil or cyclophosphamide) are sometimes used. It is usual to give six cycles of chemotherapy or four following achievement of complete remission.

More intensive chemotherapy regimens such as Stanford V or BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone) may be useful for poor-risk disease and trials to compare these with ABVD are underway.

Assessment of response to treatment

Clinical examination and imaging (e.g. CT scan) are used to assess response to treatment. Patients with Hodgkin's lymphoma often show residual masses following treatment which may be because of the large degree of fibrosis present within lymph nodes. It can be difficult to assess whether or not such masses represent residual disease and the label of complete response uncertain (CRu) can be applied in these cases. PET scanning combined with CT (see p. 211) is useful is revealing areas of active disease and further biopsy may also be required.

Relapsed cases

The patient is treated with an alternative combination chemotherapy to the initial regimen and, if necessary, with radiotherapy to sites of bulky disease. If the disease remains chemosensitive, high-dose chemotherapy and autologous stem cell transplantation improve the probability of cure and are recommended for most patients below the age of 65 years. Allogeneic transplantation may also be tried in a minority of patients who fail other therapies.

Prognosis

Approximate 5-year survival rates range from 50% to over 90% depending on age, stage and histology.

The late effects of Hodgkin's lymphoma and its treatment

Long-term follow-up of patients has revealed a considerable burden of late disease following treatment. Secondary cancers such as lung cancer and breast cancer appear to be related to radiotherapy whereas myelodysplasia or acute myeloid leukaemia are more associated with the use of alkylating agents. Non-Hodgkin's lymphomas and other cancers also occur with greater frequency than in controls. Non-malignant complications include sterility, intestinal complications, myocardial infarction and other cardiac or pulmonary complications of the mediastinal radiation and chemotherapy. These features are the main reason why less intensive treatment regimens are now being explored for this disease.

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CHAPTER 17

17 Non-Hodgkin's lymphoma

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Non-Hodgkin's lymphomas

These are a large group of lymphoid tumours, most commonly of B-cell origin, whose clinical presentation and natural history are more variable than in Hodgkin's lymphoma. They are characterized by an irregular pattern of spread and a significant proportion of patients develop extranodal disease. Their frequency has increased markedly over the last 50 years and with an incidence of approximately 17 in 100 000 they now represent the fifth most common malignancy in some developed countries. The aetiology of the majority of cases of non-Hodgkin's lymphomas (NHL) is unknown although infectious agents are an important cause in particular subtypes (Chapter 10).

Classification and histopathology

No area of diagnostic histopathology has been associated with greater confusion than the classification of NHL. A number of classifications have been used over the years with no one scheme receiving unanimous support. In 1994, the Revised American European Lymphoma (REAL) classification was published and gained widespread application. The more recent World Health Organization (WHO) classification, based on REAL, includes all lymphoid malignancies as well as lymphomas and is more clinically based than previous classification schemes (Table 17.1). In general terms, there is a move away from subdividing lymphomas on the basis of subtle histological appearance and more in terms of distinct syndromes each with characteristic morphological, immunophenotypic, genetic and clinical features. It is also useful to think of the likely origin of individual lymphoid malignancies based on their phenotype and immunoglobulin gene rearrangement status (Fig. 17.1). In this chapter we consider each of the common lymphoma subtypes within this classification.

Low- and high-grade non-Hodgkin's lymphomas

The non-Hodgkin's lymphomas are a diverse group of diseases and vary from highly proliferative and rapidly fatal diseases to some of the most indolent and well-tolerated malignancies. For many years clinicians have subdivided lymphomas into low- and high-grade disease, with some falling into an intermediate grade. This approach has been extremely valuable as, in general terms, the low-grade disorders are relatively indolent, respond well to chemotherapy but are very difficult to cure whereas high-grade lymphomas are aggressive and need urgent treatment but are often curable.

Clinical features of non-Hodgkin's lymphomas

1 *Superficial lymphadenopathy* The majority of patients present with asymmetric painless enlargement

Table 17.1 The World Health Organization (WHO) classification of non-Hodgkin's lymphomas. B-cell disorders comprise of 85% of cases. T cell and NK cell together comprise of 15% of cases.

Precursor B-cell neoplasm	*Precursor T-cell neoplasms*
B-lymphoblastic leukaemia/lymphoma (precursor B-cell ALL, B-ALL/LBL)	T-cell lymphoblastic lymphoma/leukaemia (T-ALL/LBL)
Mature B-cell neoplasms	*Mature T-cell and NK cell neoplasms*
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma	T-cell prolymphocytic leukaemia
	T-cell granular lymphocytic leukaemia
B-cell prolymphocytic leukaemia	Aggressive NK-cell leukaemia
Lymphoplasmacytic lymphoma	Adult T-cell lymphoma/leukaemia (HTLV-1+)
Splenic marginal zone B-cell lymphoma (±villous lymphocytes)	Extranodal NK/T-cell lymphoma, nasal type
Hairy cell leukaemia	Enteropathy-type T-cell lymphoma
Plasma cell myeloma/plasmacytoma	Mycosis fungoides/Sézary syndrome
	Anaplastic large cell lymphoma, primary cutaneous type
Extranodal marginal zone B-cell lymphoma of MALT type	Peripheral T-cell lymphoma, unspecified
	Angioimmunoblastic T-cell lymphoma
Mantle cell lymphoma	Anaplastic large cell lymphoma, primary systemic type
Follicular lymphoma	
Nodal marginal zone B-cell lymphoma	
Diffuse large B-cell lymphoma	
Burkitt's lymphoma/Burkitt's cell leukaemia	
Primary effusion lymphoma	
Mediastinal large B-cell lymphoma	

ALL, acute lymphoblastic leukaemia; HTLV, human T-cell leukaemia/lymphoma virus; LBL, lymphoblastic lymphoma; MALT, mucosa-associated lymphoid tissue; NK, natural killer.

of lymph nodes in one or more peripheral lymph node regions.

2 *Constitutional symptoms* Fever, night sweats and weight loss occur less frequently than in Hodgkin's disease and their presence is usually associated with disseminated disease.

3 *Oropharyngeal involvement* In 5–10% of patients there is disease of the oropharyngeal lymphoid structures (Waldeyer's ring) which may cause complaints of a 'sore throat' or noisy or obstructed breathing.

4 *Presenting features* Anaemia, neutropenia with infections or thrombocytopenia with purpura may be presenting features in patients with diffuse bone marrow disease. Cytopenias may also be autoimmune in origin. Infections may occur as a result of neutropenia or reduced cell immunity (e.g. herpes zoster).

5 *Abdominal disease* The liver and spleen are often enlarged and involvement of retroperitoneal or mesenteric nodes is frequent. The gastrointestinal tract is the most commonly involved extranodal site after the bone marrow, and patients may present with acute abdominal symptoms.

6 *Other organs* Skin, brain, testis or thyroid involvement is not infrequent. The skin is also primarily involved in two unusual, closely related T-cell lymphomas: mycosis fungoides and Sézary syndrome.

Investigations

Histology

Lymph node biopsy is the definitive investigation (Figs 17.2 and 17.3) and morphological examination is assisted by immunophenotypic and genetic analysis. For B-cell lymphomas, expression of either κ or λ light chains confirms clonality and distinquishes the disease from a reactive node (Fig. 17.4; Chapter 10).

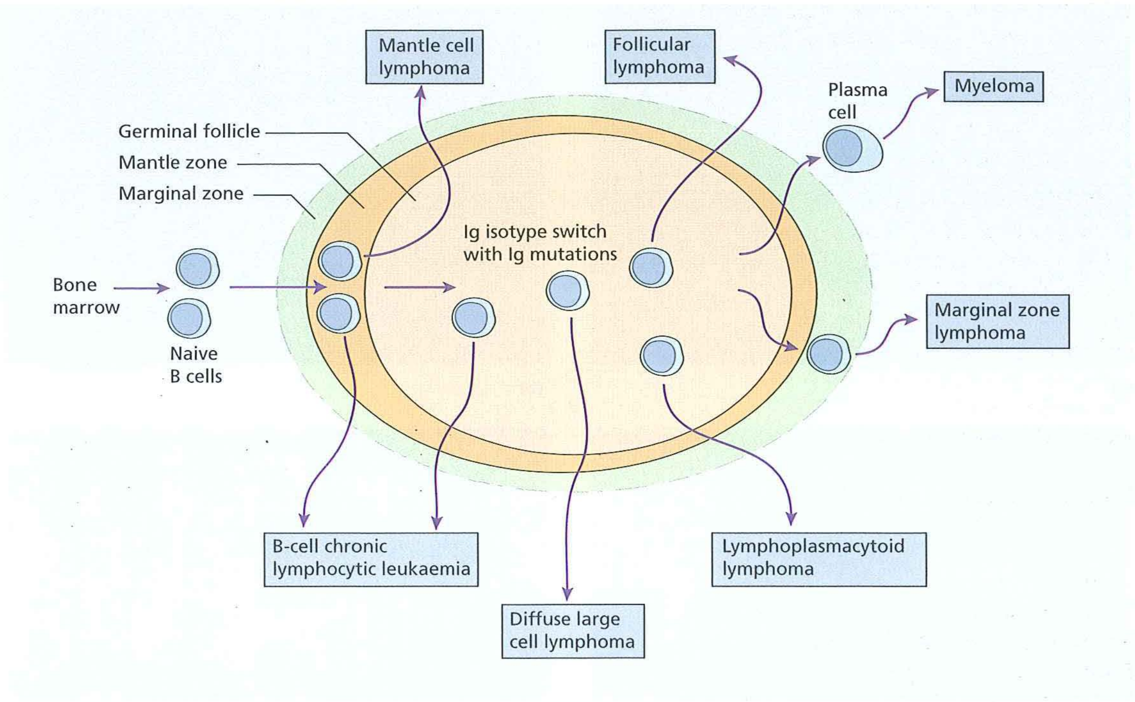


Fig. 17.1 Proposed cellular origin of B-lymphoid malignancies. Normal B cells migrate from the bone marrow and enter secondary lymphoid tissue. When they encounter antigen a germinal centre is formed and B cells undergo somatic hypermutation of the immunoglobulin genes. Finally, B cells exit the lymph node as memory B cells or plasma cells. The cellular origin of the different lymphoid malignancies can be inferred from immunoglobulin gene rearrangement status and membrane phenotype. Mantle cell lymphoma and a proportion of B-CLL cases have unmutated immunoglobulin genes whereas marginal zone lymphoma, diffuse large cell lymphoma, follicle cell lymphoma, lymphoplasmacytoid lymphoma and some B-CLL cases have mutated immunoglobulin genes.

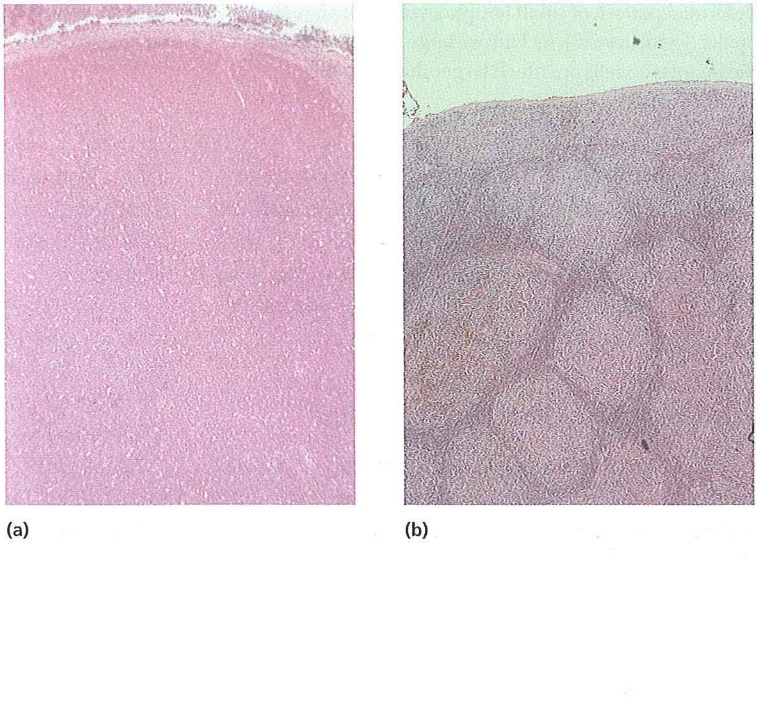


Fig. 17.2 Non-Hodgkin's lymphoma: histological sections of lymph nodes showing: **(a)** a diffuse pattern of involvement in lymphocytic lymphoma with the normal architecture totally replaced by neoplastic lymphocytic cells; **(b)** a follicular or nodular pattern in follicular lymphoma—the 'follicles' or 'nodules' of neoplastic cells compress surrounding tissue and lack a mantle of small lymphocytes.

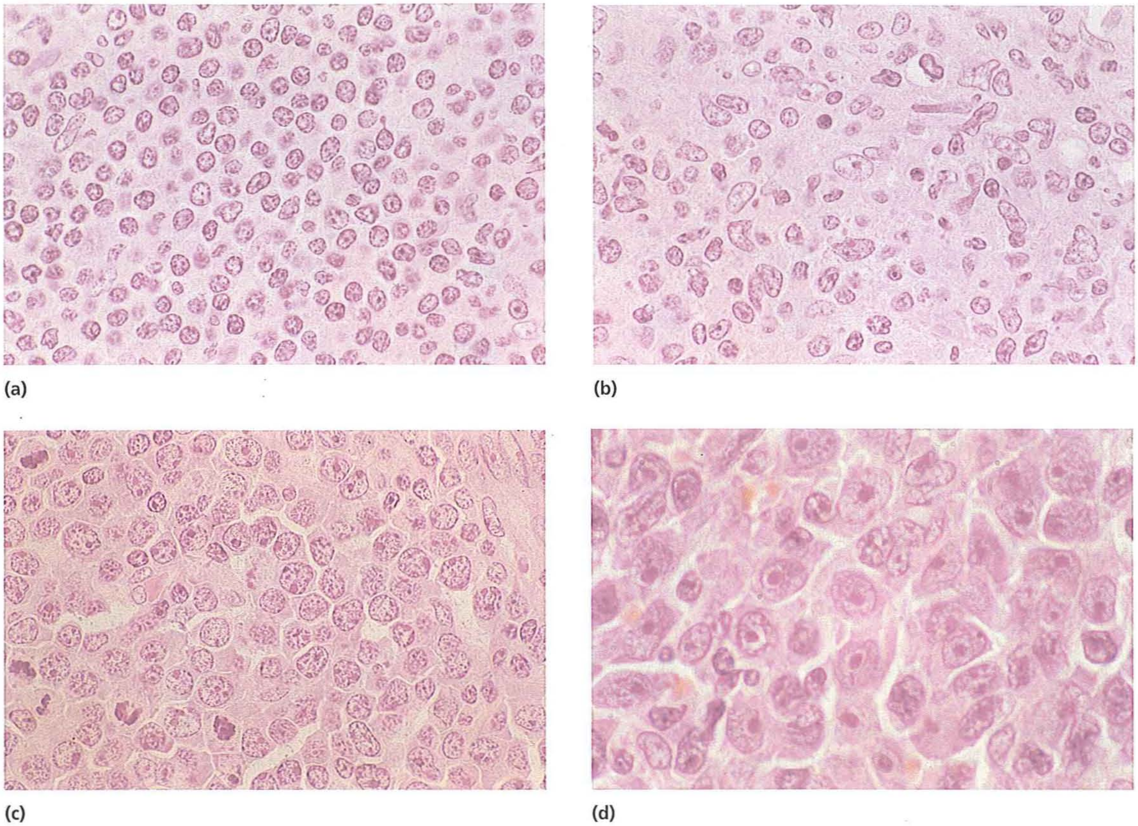


Fig. 17.3 Non-Hodgkin's lymphoma: high power view of lymph node biopsies showing **(a)** lymphocytic lymphoma showing predominantly small lymphocytes with round nuclei containing densely clumped heterochromatin. **(b)** Mantle cell lymphoma: showing characteristic deformed pattern of small lymphocytes with angular nuclei ('centrocytes'). **(c)** Diffuse large B-cell lymphoma: the neoplastic cells are much larger than normal lymphocytes and have a round nucleus with prominent nucleoli, many of which are adjacent to the nuclear membrane ('centroblasts'). A number of mitotic figures are seen. **(d)** Diffuse large B-cell lymphoma showing large neoplastic cells with a single prominent nucleolus and abundant dark-staining cytoplasm (previously termed immunoblasts).

The repertoire of CD antigens expressed on the tumour cell surface is also useful in the classification of malignant lymphoma (Table 17.2; see Appendix 1). Trephine biopsy of the marrow and immunoglobulin or T-cell receptor gene rearrangement studies may also be valuable.

Haematological and biochemical findings

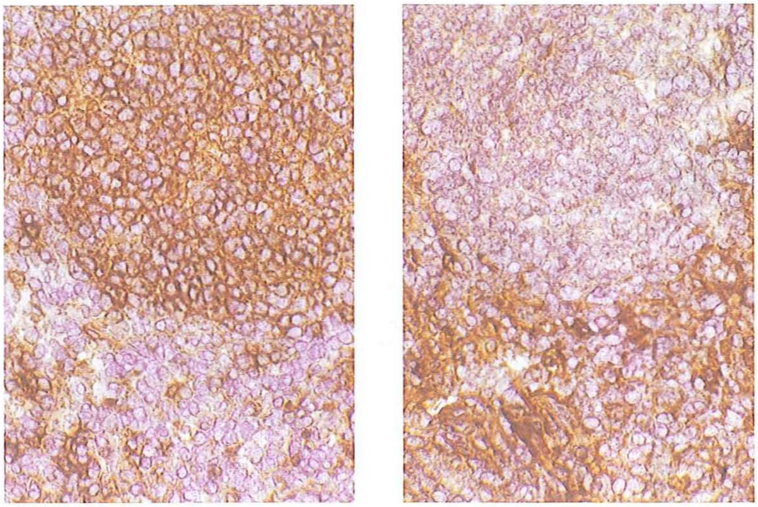
1 A normochromic, normocytic anaemia is usual but autoimmune haemolytic anaemia may also occur.

2 In advanced disease with marrow involvement there may be neutropenia, thrombocytopenia (especially if the spleen is enlarged) or leucoerythroblastic features.

3 Lymphoma cells (e.g. mantle zone cells, 'cleaved follicular lymphoma' or 'blast' cells) with variable nuclear abnormalities may be found in the peripheral blood in some patients (Fig. 15.7).

4 Trephine biopsy of marrow is valuable (Fig. 17.6). Paradoxically, bone marrow involvement is found more frequently in low-grade malignant lymphomas.

5 The serum lactate dehydrogenase (LDH) level is



(a) (b)

Fig. 17.4 Non-Hodgkin's lymphoma: lymph node stained by immunoperoxidase shows **(a)** brown ring staining for κ in the malignant lymphoid nodule, and **(b)** no labelling for λ confirming the monoclonal origin of the lymphoma.

Table 17.2 Characteristic immunophenotype and cytogenetics of B-cell lymphomas.

Lymphoma	Surface immunoglobulin	CD5	CD10	CD20	CD23	CD43	Cyclin D_1	Typical cytogenetics
CLL/lymphocytic lymphoma	Weak	+	–	+	+	+	–	13q14, 17p or 11q deletions; trisomy 12
Follicular lymphoma	+	–	+	+	+/–	–	–	t(14; 18)
Mantle cell lymphoma	+	+	–	+	–	+	+	t(11; 14)
Diffuse large cell lymphoma	+/–	–	+/–	+	–	+/–	–	various
Burkitt's lymphoma	+	–	+	+	–	–	–	t(8; 14)
MALT lymphoma	+	–	–	+	+/–	+/–	–	t(1; 14)

CLL, chronic lymphocytic leukaemia; MALT, mucosa-associated lymphoid tissue.
More detailed cytogenetics are given in Table 10.2.

raised in more rapidly proliferating and extensive disease and may be used as a prognostic marker (Table 17.3). Elevation of serum uric acid may occur.
6 Immunoglobulin electrophoresis may reveal a paraprotein.

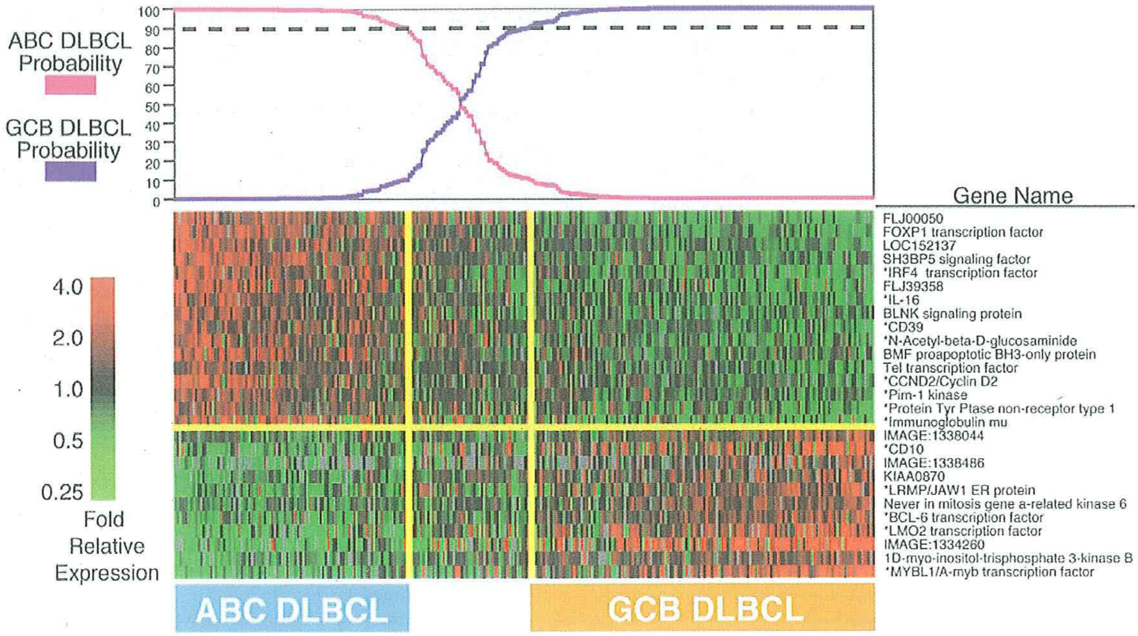
Cytogenetics

The various subtypes of NHL are associated with characteristic chromosomal translocations which are of diagnostic and prognostic value (see Tables 10.2; 17.2). Particularly characteristic translocations are

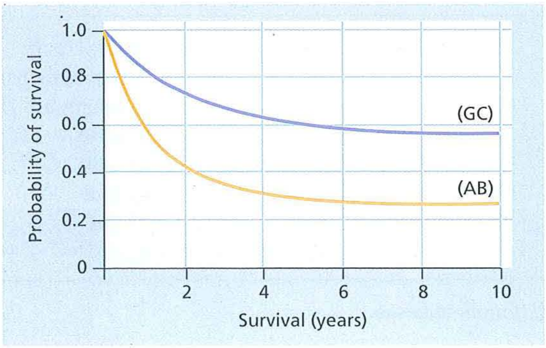
Table 17.3 International prognostic index for high-grade lymphoma.

	Good	Bad
Age	<60 years	>60 years
Performance status	0 or 1	>2
Stage (p. 200)	I or II	III or IV
Number of extranodal sites	0 or 1	>2
Serum LDH	Normal	Raised

LDH, lactate dehydrogenase.



(a)



(b)

Fig. 17.5 **(a)** DNA microarray of diffuse large B-cell lymphoma (DLCL) which uses expression analysis of 27 genes to divide cases into those with a typical activated B cell (ABC) or germinal centre B cell (GCB) pattern (Wright *et al.*, 2003). **(b)** Survival of patients with DLCL grouped according to an activated B cell (ABC) or germinal centre B cell (GCB) microarray expression pattern. (After Wright *et al.* 2003)

t(8; 14) in Burkitt's lymphoma, t(14; 18) in follicular lymphoma, t(11; 14) in mantle cell lymphoma and t(2; 5) in anaplastic large cell lymphoma.

In B-cell lymphomas the immunoglobulin genes are clonally rearranged whereas in T-cell lymphomas there is clonal rearrangement of the T-cell receptor genes (Chapter 10). DNA microarray patterns of gene expression have been shown to give valuable diagnostic and prognostic information (Fig. 17.5) but are not yet widely available.

Staging

The staging system is the same as that described for Hodgkin's disease but is less clearly related to prognosis than histological type. Staging procedures usually include chest X-ray, computed tomography (CT) scanning or magnetic resonance imaging (MRI) (Fig. 17.7) and bone marrow aspiration and trephine (Fig. 17.6). Positron-emission tomography (PET) may detect disease not seen on

Fig. 17.6 Iliac crest trephine biopsy in lymphocytic lymphoma. Prominent nodules of lymphoid tissue are seen in the intertrabecular space and paratrabecular areas.

CT scan and is useful for following treatment response (Fig. 17.7). Combined PET-CT is now commonly used in initial staging and for detection of residual disease (Fig. 17.8).

Specific subtypes of non-Hodgkin's lymphoma

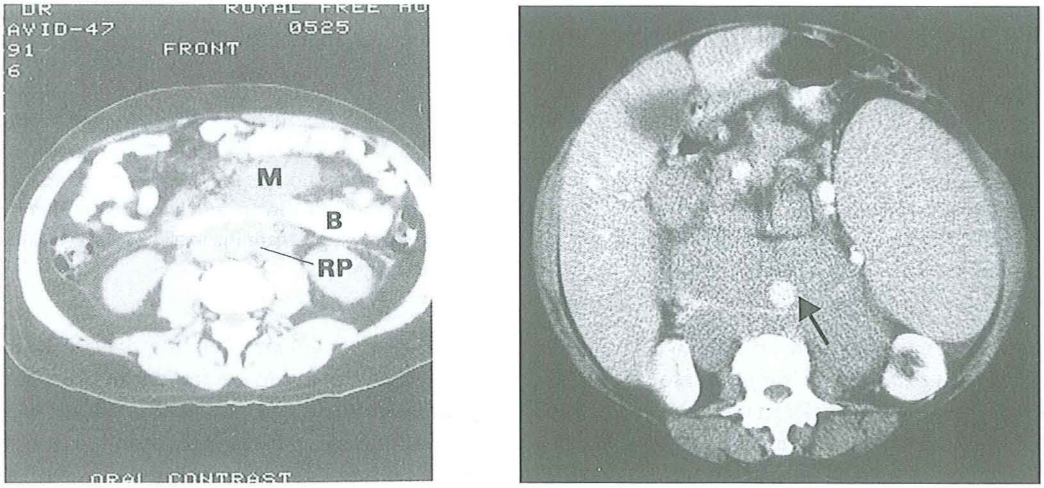
Low-grade non-Hodgkin's lymphoma

Follicular lymphoma

This is the most common form of NHL and is associated with the t(14; 18) translocation and constitutive BCL-2 expression in the great majority of cases (Fig. 17.9). Patients are likely to be middle-aged or elderly and their disease is often characterized by a benign course for many years. The median survival from diagnosis is approximately 10 years.

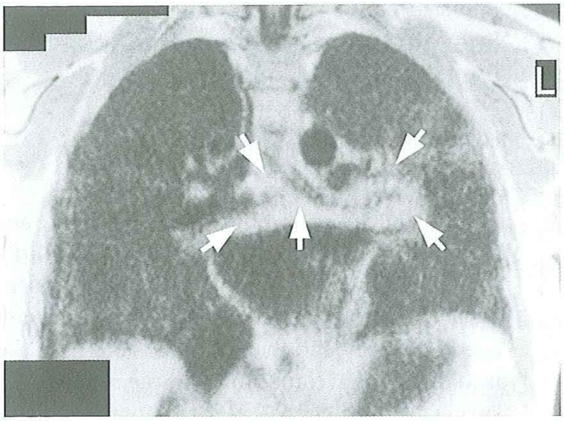
Presentation is usually with painless lymphadenopathy, often widespread, and the majority of patients will have stage III or IV disease. However, sudden transformation may occur to aggressive diffuse tumours which are sometimes associated with a leukaemic phase.

Patients with localized disease may achieve cure with radiotherapy alone. Treatment options for disseminated (stage II–IV) disease range from simple observation through oral chemotherapy to experimental high-dose treatment with stem cell support. When used as initial treatment, chlorambucil or cyclophosphamide, vincristine and prednisolone

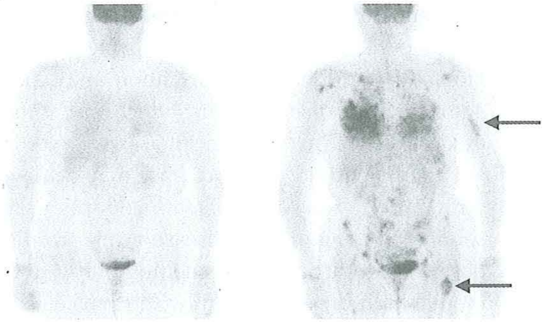


(a) (b)

Fig. 17.7 Non-Hodgkin's lymphoma. **(a)** Computed tomography (CT) scan of the abdomen showing enlarged mesenteric (M) and retroperitoneal (RP; para-aortic) lymph nodes; B, bowel. (Courtesy of Dr L. Berger) **(b)** CT scan of the abdomen: enlarged retroperitoneal and mesenteric nodes from a man causing the 'floating aorta' (arrowed) appearance. (Courtesy of Professor A. Dixon and Dr R.E. Marcus) *(Continued)*

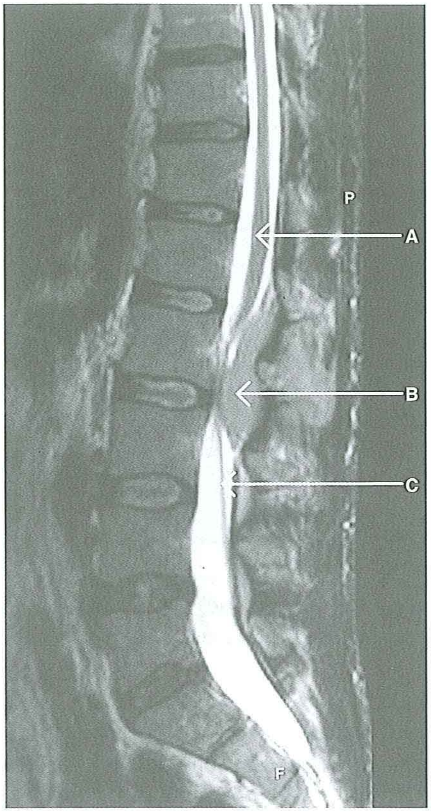


(c)



(i) (ii)

(e)



(d)

Fig. 17.7 (*Continued*) **(c)** Magnetic resonance imaging (MRI) scan of the chest showing large mediastinal lymph nodes (white and arrowed) adjacent to the great vessels (black). **(d)** MRI T_2-weighted midline saggital image of a lumbosacral spine showing compression of the dural sac by an extradural mass. A, spinal cord; B, extradural mass; C, roots of corda equina. (Courtesy of Dr A. Valentine) **(e)** Positron emission tomography (PET) body scan of a 59-year-old woman with high-grade non-Hodgkin's lymphoma. (i) The first scan showed no evidence of disease prior to allogeneic transplant. Normal physiological uptake is seen in the brain and bladder. Two months post-transplant the patient relapsed clinically with a mass on the anterior chest wall. (ii) The PET scan showed evidence of widespread relapse in nodal (para-aortic and iliac nodes) and extranodal sites including the lung and bone. The uptake in bone is clearly demonstated in the left humerus and femur (arrowed). This scan illustrates how well PET can detect both nodal and extranodal disease and allows whole body assessment at a single scanning session. (Courtesy of Dr S.F. Barrington)

(CVP) achieve a response in up to 90% of patients with a median duration of approximately 2 years. Fludarabine alone or in combination with cyclophosphamide or mitozantrone and dexamethasone (FMD) is another option. Rituximab, a humanized monoclonal antibody to CD20, is also effective and may be used alone or combined with chemotherapy (e.g. R-CVP; Fig. 17.10). Early studies suggest it is also valuable in maintenance.

Disease relapse is initially treated with similar chemotherapy regimens but the disease becomes increasingly difficult to control and more intensive chemotherapy and radiolabelled antibody therapy may be considered. Autologous stem cell transplantation (SCT) may be valuable in patients with a history of at least one relapse and allogeneic SCT offers the prospect of cure for younger patients.

Lymphocytic lymphomas

Lymphocytic lymphomas are closely related to

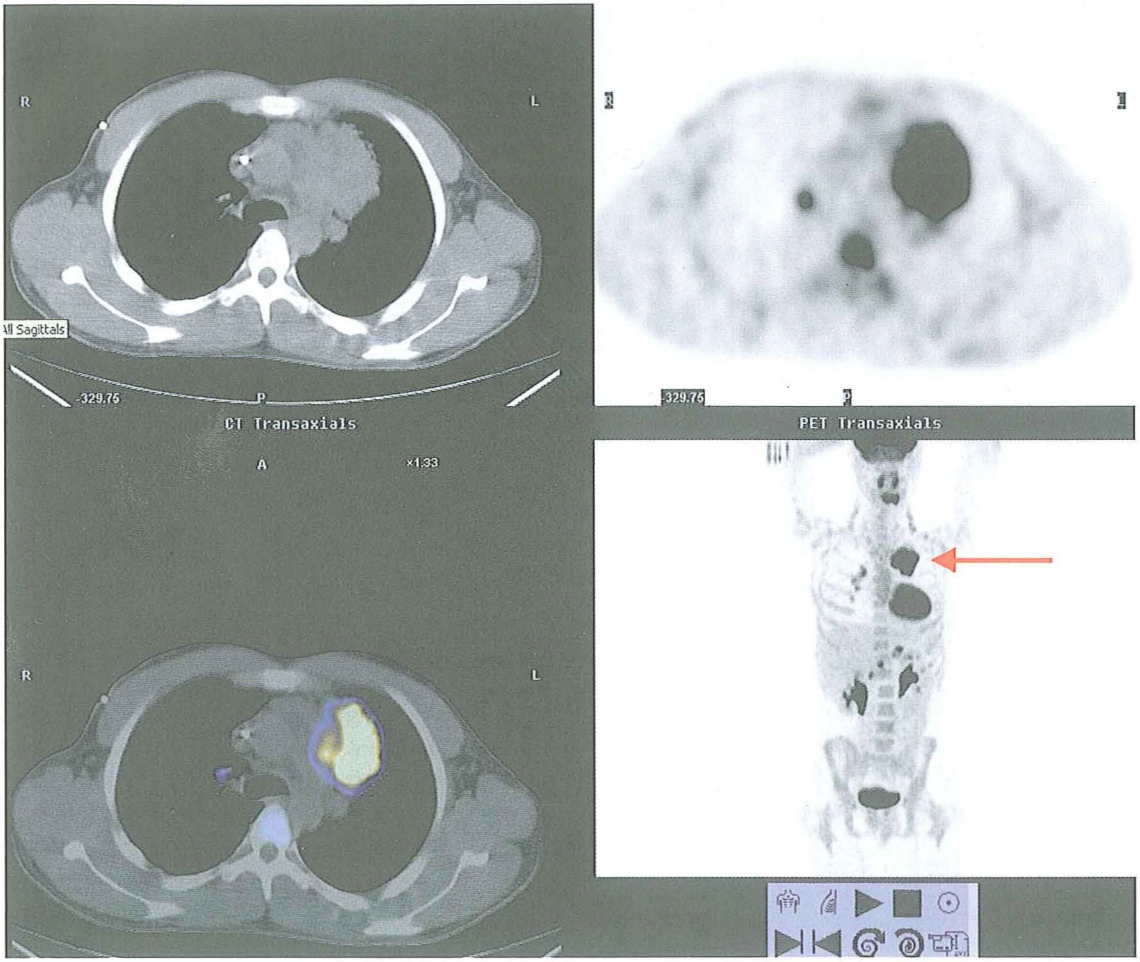


Fig. 17.8 Non-Hodgkin's lymphoma. PET/CT scan of male aged 26 years. Red arrow shows the level at which transaxial section is performed. Upper right-hand panel showing FDG uptake in anterior mediastinal mass and right hilar node. Upper left-hand panel shows corresponding CT section, bottom left-hand panel shows the CT and PET fused image of the corresponding section. Bottom right-hand panel, coronal section showing FDG uptake in mediastinal mass and right hilar node. There is also FDG uptake in the thyroid, heart, bowel, kidneys and bladder. (Courtesy of the Department of Nuclear Medicine, University College, London)

chronic lymphocytic leukaemia (CLL) and this lymphoma appears to be a tissue phase of CLL (Figs 17.2 and 17.3). Many patients with this condition are elderly with slowly progressive disease and may not require treatment for extended periods. Treatment is based on that used for CLL (p. 190).

Lymphoplasmacytoid lymphomas

Lymphoplasmacytoid lymphomas are often associated with the production of monoclonal immunoglobulin M (IgM), in which case they tend to be termed Waldenström's macroglobulinaemia (p. 223). Complications are anaemia and hyperviscosity syndrome. Treatment is with oral chlorambucil, fludarabine or monoclonal antibodies (e.g. rituximab). Plasma exchanges may be needed to reduce hyperviscosity.

Mantle cell lymphoma

Mantle cell lymphoma is derived from pre-germinal centre cells localized in the primary follicles or in the mantle region of secondary follicles. It has a

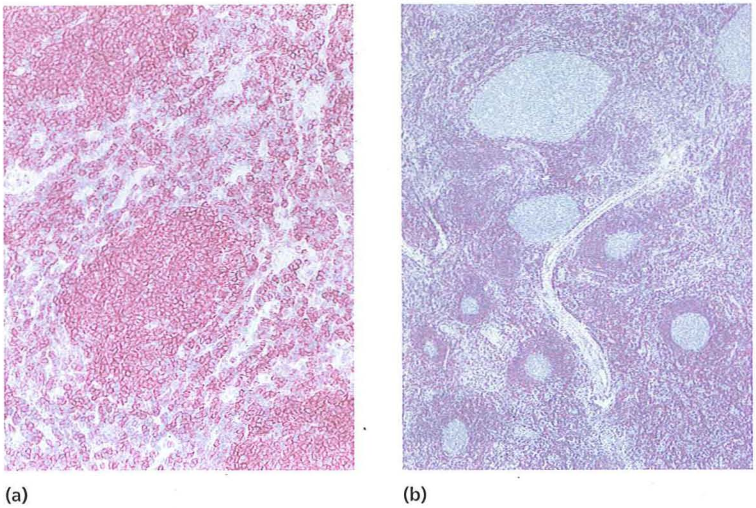


Fig. 17.9 Follicular lymphoma: immunohistological detection of BCL-2 protein. (a) Follicular lymphoma positive because the BCL-2 is activated by the (14; 18) translocation; (b) reactive node shows germinal centres unstained for BCL-2 surrounded by positive mantle zone B and T cells. Immunoalkaline phosphatase (APAPP) stain. (Courtesy of Professors K.C. Gatter and D.Y. Mason)

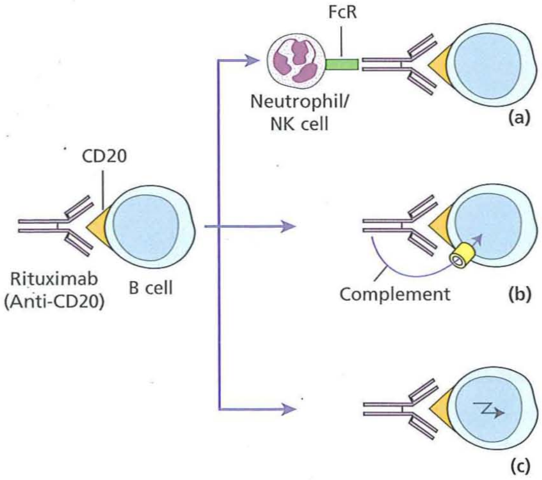


Fig. 17.10 The potential mechanisms of action of rituximab. Rituximab binds to CD20 on the surface of B cells. It can elicit a number of effector mechanisms including: **(a)** antibody dependent cell-mediated cytotoxicity; **(b)** complement mediated lysis of tumour cells; and **(c)** direct apoptosis of the target cell.

characteristic phenotype of $CD19^+$ and $CD5^+$ (like CLL) but in contrast is $CD22^+$, $CD23^-$. A specific t(11; 14) (q13; q32) translocation is seen which leads to deregulation of the cyclin D_1 (*BCL-1*) gene by juxtaposing it with the immunoglobulin heavy-chain gene. Clinical presentation is typically with lymphadenopathy and often there is bone marrow infiltration and tumour cells in the blood. The cells have characteristically angular nuclei in histological sections (Fig. 17.3b) and may circulate in the blood (see Fig. 15.6). Despite its low-grade status, mantle cell lymphoma is characterized by a poor prognosis. Current treatment regimens such as CVP, cyclophosphamide, hydroxodaunorubicin, oncovin (vincristine) and prednisolone (CHOP) or fludarabine, each with rituximab, are used. The role of bortezomid (see p. 223) or autologous stem cell transplantation is being investigated. The prognosis is poor and the median survival is approximately 3 years.

Marginal zone lymphomas

Marginal zone lymphomas are typically extranodal and are usually localized. Mucosa-associated lymphoid tissue (MALT) lymphomas come into this category and usually arise as a consequence of a pre-existing inflammatory or autoimmune disorder at sites such as the stomach or thyroid. Gastric MALT lymphoma is the most common form and is preceded by *Helicobacter (H.) pylori* infection. In the early stages it may respond to antibiotic therapy aimed at eliminating *H. pylori*. Chemotherapy and/or radiotherapy combined with rituximab as for other low-grade lymphoma may be needed.

Splenic marginal zone lymphoma usually presents as splenomegaly and may be associated with circulating 'villous' lymphocytes (p. 194). Splenectomy is useful for symptomatic patients.

High-grade non-Hodgkin's lymphoma

Diffuse large B-cell lymphomas

Diffuse large B-cell lymphomas (DLCL) are a heterogeneous group of disorders representing the classical 'high-grade' lymphomas. As such they typically present with rapidly progressive lymphadenopathy associated with a fast rate of cellular proliferation. Progressive infiltration may affect the bone marrow, gastrointestinal tract, the spinal cord, the kidneys or other organs.

A variety of clinical and laboratory findings are relevant to the outcome of therapy. According to the international prognostic index these include age, performance status, stage, number of extranodal sites and serum LDH (Table 17.3). Bulky disease (major mass >5 cm in diameter) and prior history of low-grade disease or AIDS are also associated with a poorer prognosis. The cell of origin of DLCL has recently been suggested to be of prognostic significance. If this is germinal centre the outlook has been found to be more favourable than if the origin is from an activated peripheral B cell. Cases associated with 3q27 translocation also have a relatively good prognosis. DNA microarray analysis can give prognostic information (Fig. 17.5).

The CHOP regimen has been the mainstay of treatment of DLCL for many years and consists of cyclophosphomide, hyroxodaunorubicin, vincristine (Oncovin) and prednisolone given in 2-, 3- or 4-weekly cycles, typically for six to eight courses. The addition of anti-CD20 (rituximab) improves the cure rate and so R-CHOP is now the initial treatment. Cycles given at 14-day intervals with G-CSF support are under trial in younger patients. For localized disease, combined radiotherapy and chemotherapy (e.g. three courses of R-CHOP) may be optimal. Prophylactic therapy to prevent central nervous system (CNS) disease such as intrathecal or high-dose systemic methotrexate should be considered for patients with high-risk disease, particularly those with bone marrow involvement.

For patients who relapse and have chemotherapy-sensitive disease, high-dose chemotherapy with ifosfamide, epirubicin and etoposide (IVE) or etoposide, cytosine arabinoside, methylprednisolone and cisplatin (ESHAP), followed by autologous SCT can be effective. For those with primary refractory or chemoresistant disease the outlook is poor. Bortezomib, a proteasome inhibitor is undergoing trials. Alemtuzumab directed against CD52 may be used in both B- and T-cell NHL. Anti-CD20 radioactively linked to yttrium-90 or iodine-131 is currently reserved for relapsed cases. Overall long-term survival is approximately 65%.

Burkitt's lymphoma

Burkitt's lymphoma is the lymphomatous correlate of L_3 acute lymphoblastic leukaemia (p. 159) and occurs in endemic or sporadic forms. Endemic (African) Burkitt's lymphoma is seen in areas with chronic malaria exposure and is associated with Epstein–Barr virus (EBV) infection. In virtually all cases the *C-MYC* oncogene is overexpressed because it is translocated to an immunoglobulin gene, usually the heavy-chain locus t(8; 14). Typically, the patient, usually a child, presents with massive lymphadenopathy of the jaw (Fig. 17.11) which is initially very responsive to chemotherapy although long-term cure is uncommon. Sporadic Burkitt's lymphoma may occur anywhere in the world and is not associated with EBV infection. The histological picture of Burkitt's lymphoma is distinctive (Fig. 17.12). The prognosis for such patients was poor until the introduction of chemotherapy

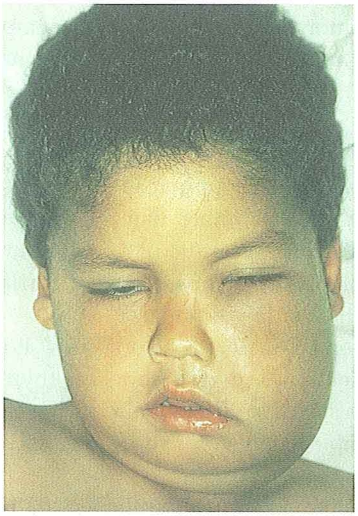


Fig. 17.11 Burkitt's lymphoma: characteristic facial swelling caused by extensive tumour involvement of the mandible and surrounding soft tissues.

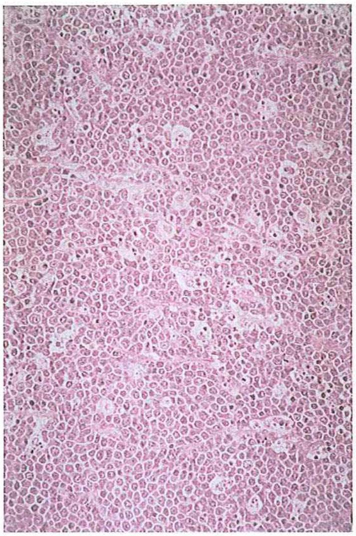


Fig. 17.12 Burkitt's lymphoma: histological section of lymph node showing sheets of lymphoblasts and 'starry sky' tingible body macrophages.

regimes which include high-dose methotrexate and cyclophosphamide. These have transformed the outlook and now the majority of patients are cured. B-ALL is treated with the same regimes.

Lymphoblastic lymphomas

Lymphoblastic lymphomas occur mainly in children and young adults and these conditions merge clinically and morphologically with acute lymphoblastic leukaemia (ALL). They are treated in a similar way.

T-cell lymphomas

Peripheral T-cell lymphomas that present with lymphadenopathy rather than extranodal disease are a heterogeneous group of rare tumours and are usually of $CD4^+$ phenotype. Several variants of T-cell lymphomas are recognized.

Peripheral T-cell non-Hodgkin's lymphoma, unspecified. These derive from post-thymic T cells at various stages of differentiation. They are treated with combined chemotherapy (e.g. CHOP). The prognosis is poor.

Angioimmunoblastic lymphadenopathy usually occurs in elderly patients with lymphadenopathy, hepatosplenomegaly, skin rashes and a polyclonal increase in serum IgG.

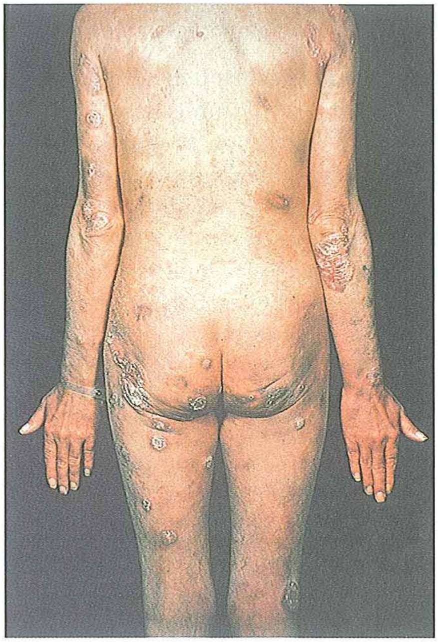


Fig. 17.13 Mycosis fungoides.

Mycosis fungoides is a chronic cutaneous T-cell lymphoma that presents with severe pruritus and psoriasis-like lesions (Fig. 17.13). Ultimately, deeper organs are affected, particularly lymph nodes, spleen, liver and bone marrow.

In **Sézary syndrome** there is dermatitis, erythroderma, generalized lymphadenopathy and circulating T-lymphoma cells. The cells are usually $CD4^+$ and have a folded or cerebriform nuclear chromatin. Initial treatment of these conditions is by local irradiation, topical chemotherapy or photochemotherapy with psoralen and ultraviolet light (PUVA). Chemotherapy (e.g. CHOP) may be needed.

Adult T-cell leukaemia/lymphoma is associated with human T-cell leukaemia/lymphoma virus type 1 (HTLV-1) infection and presents with lymphadenopathy, hepatic and splenic enlargement, cutaneous infiltrations and hypercalcaemia.

Angiocentric lymphomas typically involve the nasal sinuses whereas T-cell intestinal lymphoma is associated with gluten-induced enteropathy in many cases.

Anaplastic large cell lymphoma is particularly common in children and is of T-cell or null cell

phenotype. The disease is $CD30^+$ and associated with the t(2; 5) (p23; q35) translocation. It has an aggressive course characterized by systemic symptoms and extranodal involvement.

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CHAPTER 18

18 Multiple myeloma and related disorders

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Paraproteinaemia

This term refers to the presence of a monoclonal immunoglobulin band in the serum. Normally, serum immunoglobulins are polyclonal and represent the combined output from millions of different plasma cells. A monoclonal band (M-protein), or ***paraprotein***, reflects the synthesis of immunoglobulin from a single clone of plasma cells. There are several situations in which this may occur (Table 18.1) and not all require treatment.

Table 18.1 Diseases associated with M-proteins.

Malignant or uncontrolled production
Multiple myeloma
Waldenström's macroglobulinaemia
Malignant lymphoma
Chronic lymphocytic leukaemia
Primary amyloidosis
Plasma cell leukaemia
Heavy chain disease
Benign or stable production
Benign monoclonal gammopathy
Solitary plasmacytoma
Chronic cold haemagglutinin disease
Transient (e.g. with infections)
Acquired immune deficiency syndrome (AIDS)
Gaucher's disease
Rarely with carcinoma and other conditions

Multiple myeloma

Multiple myeloma (myelomatosis) is a neoplastic proliferation characterized by plasma cell accumulation in the bone marrow, the presence of monoclonal protein in the serum and/or urine and related tissue damage. Ninety-eight per cent of cases occur over the age of 40 years with a peak incidence in the seventh decade.

The myeloma cell is a post-germinal centre plasma cell that has undergone immunoglobulin class switching and somatic hypermutation and secretes the paraprotein that is present in serum. Plasma cells naturally home to the bone marrow and this characteristic is retained by the tumour cell. The aetiology of the disease is unknown but it is more common in certain racial groups such as those of Afro-Caribbean origin. Tumour cells have very complex genetic changes but dysregulated or increased expression of cyclin D (p. 9) is believed to be an early unifying event. Approximately half of the tumours have an increased number of chromosomes (*hyperdiploid*) whereas non-hyperdiploid cases have a high incidence of translocations involving the immunoglobulin heavy-chain gene (*IGH*). Monoallelic loss of 13q is frequent in both categories and all these genetic abnormalities are also seen in monoclonal gammopathy of undetermined significance (MGUS; p. 224). The characteristic immunophenotype of malignant plasma cells is $CD38^{high}$,

Table 18.2 Features of benign and malignant paraproteinaemia.

	Benign	**Malignant**
Bence-Jones proteinuria	Absent	May be present
Serum paraprotein concentration	Usually <20 g/L and stationary	Usually >20 g/L and rising
Serum free light chain ratio	Normal	Abnormal
Immuneparesis (hypogammaglobinaemia)	Absent	Present
Underlying lymphoproliferative disease or myeloma	Absent	Present
Bone lesions	Absent	Present
Plasma cells in marrow	<10%	>10%

$CD138^{high}$ and $CD45^{low}$. Interleukin 6 is a potent growth factor for myeloma cells and is often active by an autocrine mechanism.

Diagnosis

This depends on three principal findings (Table 18.2):

1 Monoclonal protein in serum and/or urine (Fig. 18.1).

2 Increased plasma cells in the bone marrow (Fig. 18.2).

3 Related organ or tissue impairment such as bone disease, renal impairment, anaemia, hypercalaemia, hyperviscosity, amyloidosis or recurrent infection.

If the bone marrow plasma cell count is >10% but there is **no** evidence of tissue damage the disease is termed ***asymptomatic*** or ***smouldering myeloma***.

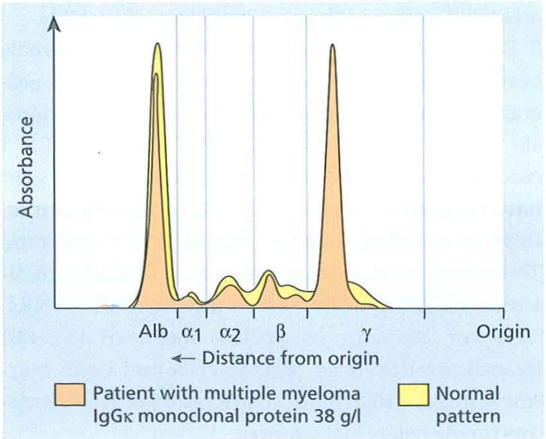


Fig. 18.1 Serum protein electrophoresis in multiple myeloma showing an abnormal paraprotein in the γ-globulin region with reduced levels of background β- and γ-globulins.

Clinical features

1 Bone pain (especially backache) resulting from vertebral collapse and pathological fractures (Fig. 18.3a,b).

2 Features of anaemia: e.g. lethargy, weakness, dyspnoea, pallor, tachycardia.

3 Recurrent infections: related to deficient antibody production, abnormal cell-mediated immunity and neutropenia.

4 Features of renal failure and/or hypercalcaemia: polydipsia, polyuria, anorexia, vomiting, constipation and mental disturbance.

5 Abnormal bleeding tendency: myeloma protein may interfere with platelet function and coagulation factors; thrombocytopenia occurs in advanced disease.

6 Amyloidosis occurs in 5% with features such as macroglossia, carpal tunnel syndrome and diarrhoea.

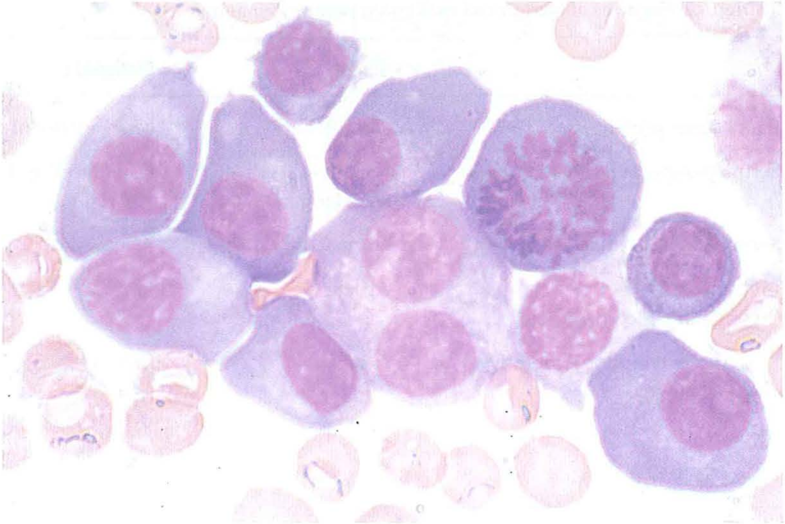


Fig. 18.2 The bone marrow in multiple myeloma showing large numbers of plasma cells, with many abnormal forms.

7 In approximately 2% of cases there is a hyperviscosity syndrome (Fig. 22.6) with purpura, haemorrhages, visual failure, central nervous system (CNS) symptoms, neuropathies and heart failure.

Laboratory findings include the following:

1 *Presence of a paraprotein* Serum and urine should be screened by immunoglobulin electrophoresis. The paraprotein is immunoglobulin G (IgG) in 60% of cases, IgA in 20% and light chain only in almost all the rest. Less than 1% have IgD or IgE paraprotein. Free light chains are filtered from the serum into the kidney and are therefore often not detectable in serum using electrophoresis. However, the level of free light chain in serum can be measured with a specific antibody. As free light chains are produced by almost all malignant plasma cells the *serum free light chain* assay is useful in diagnosis and monitoring of myeloma and other forms of paraproteinaemia. In myeloma the normal $\kappa : \lambda$ free light chain ratio of 2 : 1 is skewed with an excess of κ or λ chains.

Normal serum immunoglobulin levels (IgG, IgA and IgM) are reduced, a feature known as *immune paresis*. The urine contains free light chains, known as *Bence-Jones protein*, in two-thirds of cases. Rare cases of myeloma are non-secretory and therefore not associated with a paraprotein or Bence-Jones proteinuria although some will still show a disturbed free light chain ratio in the serum.

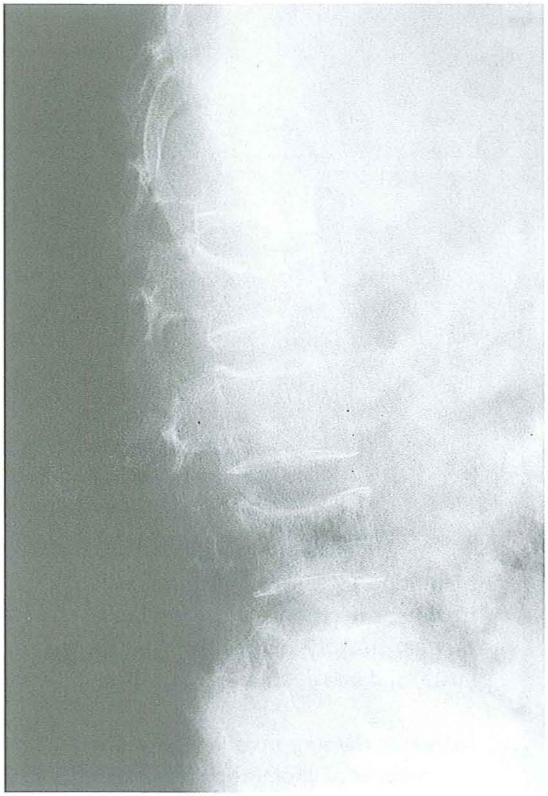
2 There is usually a normochromic normocytic or macrocytic anaemia. Rouleaux formation is marked in most cases (Fig. 18.4). Neutropenia and thrombocytopenia occur in advanced disease. Abnormal plasma cells appear in the blood film in 15% of patients and can be detected by sensitive flow cytometry in over 50%.

3 High erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

4 Increased plasma cells in the bone marrow (usually >20%) often with abnormal forms (Fig. 18.2).

5 Radiological investigation of the skeleton reveals bone lesions such as osteolytic areas without evidence of surrounding osteoblastic reaction or sclerosis in 60% of patients (Fig. 18.5) or generalized osteoporosis in 20% (Fig. 18.3). Twenty per cent have no bone lesions. In addition, pathological fractures or vertebral collapse (Fig. 18.3b) are common. The osteolytic lesions are caused by osteoclast activation resulting from high serum levels of RANKL (receptor activator of nuclear factor-κB (NF-κB) ligand), produced by plasma cells and bone marrow stroma, which binds to activatory RANK receptors on the osteoclast surface.

6 Serum calcium elevation occurs in 45% of patients. Typically, the serum alkaline phosphatase is normal (except following pathological fractures).



(a)

(b)

Fig. 18.3 (a) Multiple myeloma: X-ray of lumbar spine showing severe demineralization with partial collapse of L_3. **(b)** Magnetic resonance imaging (MRI) of spine: T_2-weighted study. There is infiltration and destruction of L_3 and L_5 with bulging of the posterior part of the body of L_3 into the spinal canal compressing the corda equina (arrowed). Radiotherapy has caused a marrow signal change in vertebrae C_2–D_4 because of replacement of normal red marrow by fat (bright white signal). (Courtesy of Dr A. Platts)

7 The serum creatinine is raised in 20% of cases. Proteinaceous deposits from heavy Bence-Jones proteinuria, hypercalcaemia, uric acid, amyloid and pyelonephritis may all contribute to renal failure (Fig. 18.6).

8 A low serum albumin occurs with advanced disease.

9 Serum β_2-microglobulin is often raised and is a useful indicator of prognosis. Levels less than 4 mg/L imply a relatively good prognosis.

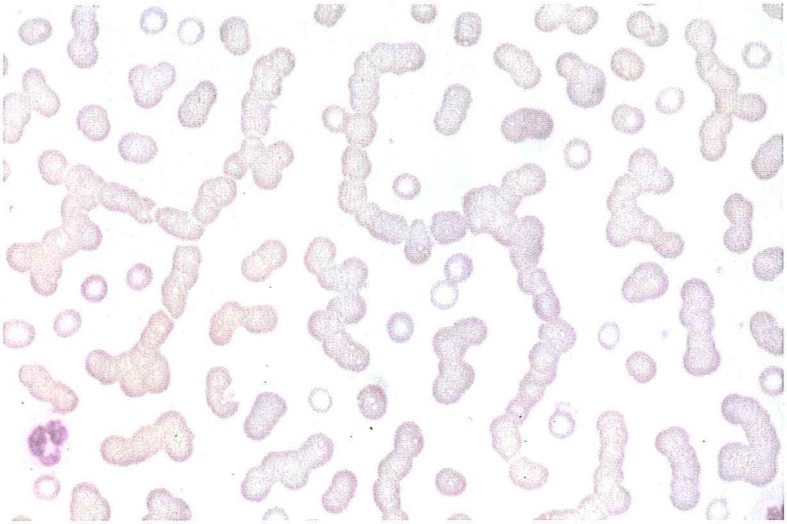


Fig. 18.4 The peripheral blood film in multiple myeloma showing rouleaux formations.

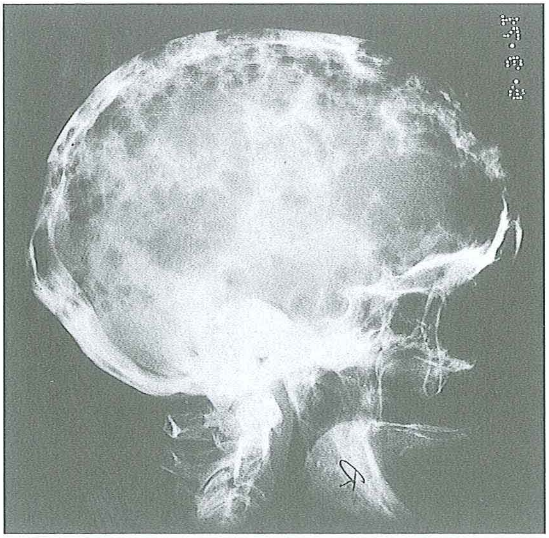


Fig. 18.5 Skull X-ray in multiple myeloma showing many 'punched-out' lesions.

Treatment

This may be divided into specific and supportive (Fig. 18.7).

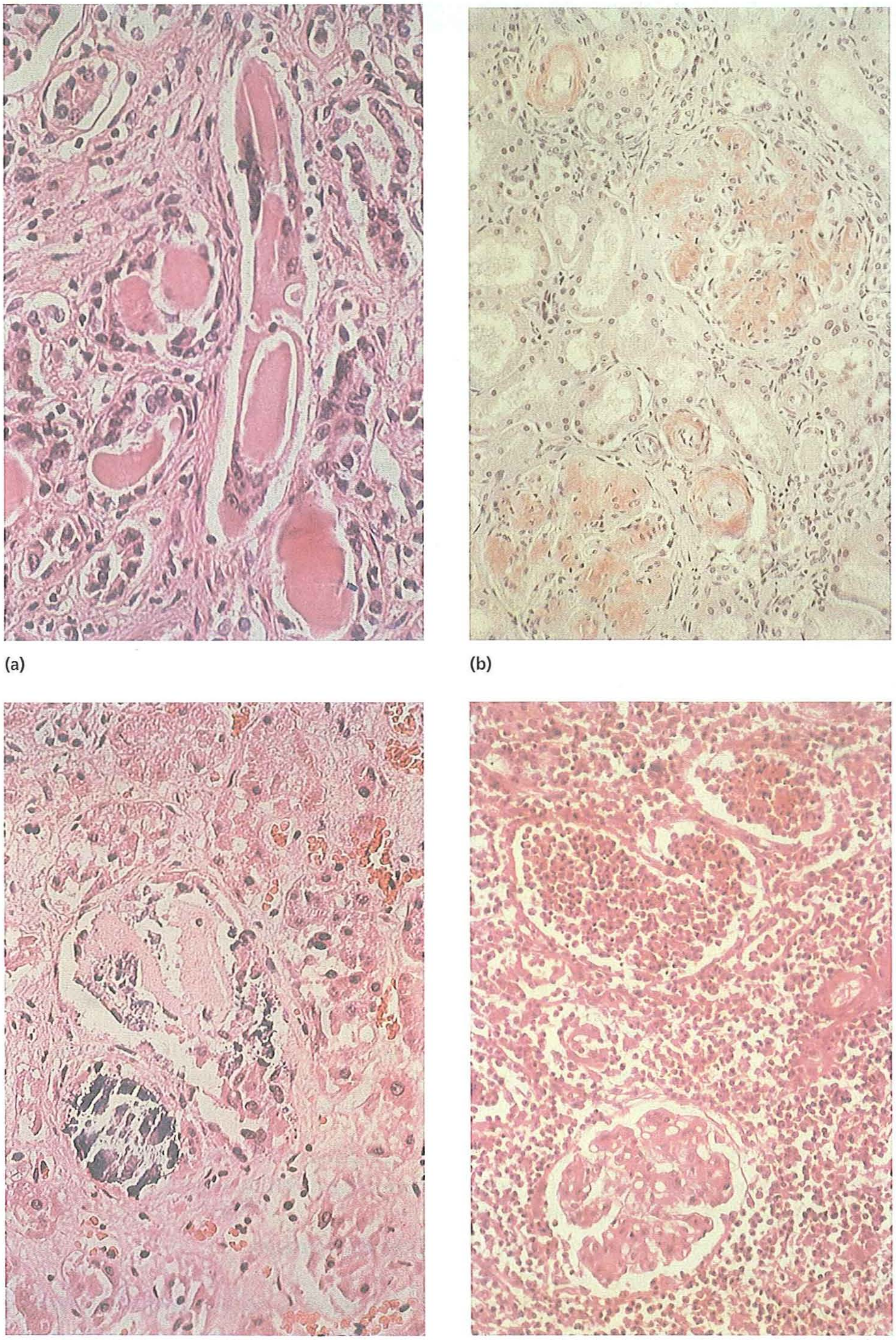
Specific

At the current time the disease remains incurable except for those very few, mostly younger, patients who may be cured by allogeneic stem cell transplantation (SCT). For all other patients the major treatment decision is between the use of ***intensive therapy*** (mostly for patients aged less than 65–70 years) and ***non-intensive therapy*** for older patients.

Intensive therapy involves the combination of several courses of chemotherapy to reduce the tumour burden followed by stem cell collection and autologous SCT after high-dose chemotherapy. Repeated intravenous or oral chemotherapy cycles such as cyclophosphamide, dexamethasone and thalidomide (CDT), cyclophosphamide, vincristine, adriamycin (hydroxodaunorubicin) and dexamethasone (CVAD) or idarubicin with dexamethasone (IDEX) may be used. Peripheral blood stem cells are collected after mobilization using a combination of chemotherapy and granulocyte colony-stimulating factor (G-CSF). High-dose melphalan, with or without radiotherapy, is the typical conditioning regime for autologous SCT. Two consecutive SCT procedures are used in some centres.

Although allogeneic transplantation may cure the disease it carries a high procedure-related mortality and patients frequently relapse after the procedure.

Non-intensive therapy In elderly patients monthly courses of the oral alkylating agent melphalan, sometimes in combination with prednisolone, are usually effective in reducing the tumour burden. Many centres now also use thalidomide (see below).



(a) (b) (c) (d)

Fig. 18.6 The kidney in multiple myeloma. **(a)** Myeloma kidney: the renal tubules are distended with hyaline protein (precipitated light chains or Bence-Jones protein). Giant cells are prominent in the surrounding cellular reaction. **(b)** Amyloid deposition: both glomeruli and several of the small blood vessels contain an amorphous pink-staining deposit characteristic of amyloid (Congo red stain). **(c)** Nephrocalcinosis: calcium deposition (dark 'fractured' material) in the renal parenchyma. **(d)** Pyelonephritis: destruction of renal parenchyma and infiltration by acute inflammatory cells.

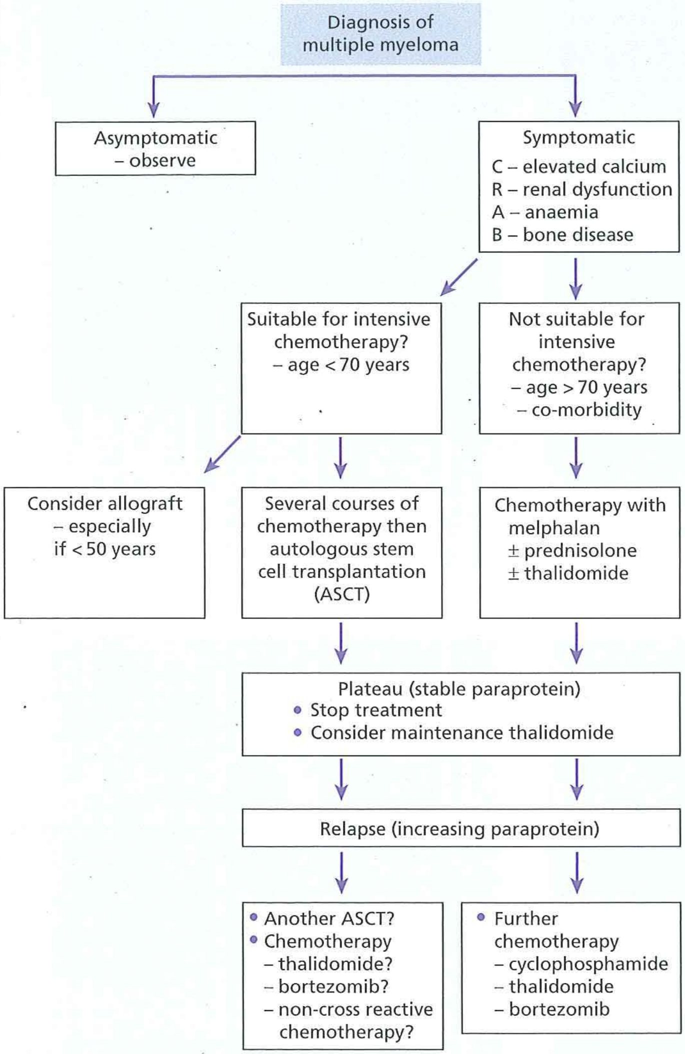


Fig. 18.7 An approach to the management of multiple myeloma.

Typically, paraprotein levels gradually fall, bone lesions show improvement and blood counts may improve. Cyclophosphamide is also effective and simple to use as a single agent. However, after a variable number of courses a 'plateau phase' is reached in which the paraprotein level stops falling. At this point treatment is stopped and the patient is seen at regular intervals in the outpatient clinic. After a variable period of time, often around 18 months, the disease 'escapes' from plateau with rising paraprotein and worsening symptoms. Further chemotherapy may be given although the disease becomes increasingly difficult to control. Trials of drugs designed to overcome multidrug resistance have not proven successful.

Other treatments

Thalidomide is useful in the management of relapsed disease and is being introduced into first-line therapy (e.g. CDT). Its precise mechanism of action is unknown and it has a number of side-effects such as sedation, constipation, neuropathy and thrombosis. The addition of dexamethasone increases the response rate but venous thrombosis becomes a major concern. Prophylactic anticoagulation with

warfarin or heparin is needed when thalidomide is used in induction regimes. Analogues of thalidomide which have increased immunomodulatory activity are now showing promise.

Bortezomib (Velcade) is a promising drug which inhibits cellular proteasome and NF-κB activation. Already proven in refractory disease, it is now being assessed in earlier phases of treatment. Its main side-effect is neuropathy.

α-Interferon This may prolong the plateau phase following chemotherapy or transplantation but has little effect on overall survival.

Radiotherapy is highly effective in treating the symptoms of myeloma. It may be used for areas of bone pain or spinal cord compression.

Support care

Renal failure Rehydrate and treat the underlying cause (e.g. hypercalcaemia, hyperuricaemia). Dialysis is generally well tolerated. It is important that all patients with myeloma drink at least 3 L of fluid each day throughout the course of their disease.

Bone disease and hypercalcaemia Bisphosphonates such as pamidronate, clodronate or zoledronic acid are effective in reducing the progression of bone disease and may also improve overall survival. Acute hypercalcaemia is treated with rehydration with isotonic saline, a diuretic and corticosteroids followed by a biphosphonate.

Compression paraplegia Use decompression laminectomy or irradiation; corticosteroid therapy may help.

Anaemia Transfusion or erythropoietin are used.

Bleeding Bleeding caused by paraprotein interference with coagulation and hyperviscosity syndrome may be treated by repeated plasmapheresis.

Infections Rapid treatment of any infection is essential. Prophylactic infusions of immunoglobulin concentrates together with oral broad-spectrum antibiotics and antifungal agents may be needed for recurrent infections.

Prognosis

An international prognostic index has been used based on serum β_2-microglobulin (β_2M) and albumin levels. Patients with serum β_2M >5.5 mg/L and an albumin <35 g/L have a poor survival as do those with frequent circulating plasma cells. Overall, the median survival with non-intensive chemotherapy is 3–4 years and this is improved by approximately 1 year with autologous transplantation.

Other plasma cell tumours

Solitary plasmacytoma

These are isolated plasma cell tumours, usually of bone or soft tissue (e.g. the mucosa of the upper respiratory and gastrointestinal tracts or the skin). The associated paraprotein disappears following radiotherapy to the primary lesion.

Plasma cell leukaemia

This occurs either as a late complication of myeloma or as a primary disease characterized by the presence of 20% or more plasma cells in the blood (Chapter 15). The outlook is poor.

Heavy chain disease

In these rare disorders the neoplastic cells secrete only incomplete immunoglobulin heavy chains (γ, α or μ). The most common form is α-heavy chain disease which occurs mainly in the Mediterranean area and starts as a malabsorption syndrome which may progress to lymphoma.

Waldenström's macroglobulinaemia

This is an uncommon condition, seen most frequently in men over 50 years of age, in which there is a lymphoplasmacytoid lymphoma (Fig. 18.8) which produces a monoclonal IgM paraprotein. The cell of origin appears to be a post-germinal centre B cell with the characteristics of an IgM-bearing memory B cell.

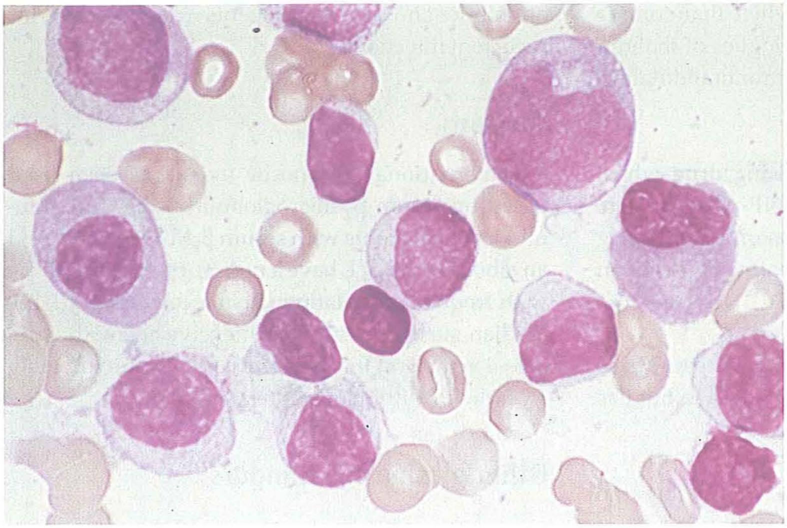


Fig 18.8 Lymphoplasmacytoid lymphoma associated with Waldenström's macroglobulinaemia. Bone marrow shows cells with features of lymphocytes and plasma cells.

Clinical features

These are usually of insidious onset, with fatigue and weight loss. Hyperviscosity syndrome (Fig. 22.6) is common. IgM paraprotein increases blood viscosity more than equivalent concentrations of IgG or IgA, and small increases above 30 g/L in concentration lead to large increases in viscosity. Visual upset is frequent and the retina may show a variety of changes such as engorged veins, haemorrhages, exudates and a blurred disc (Fig. 18.9). If the macroglobulin is a cryoglobulin, features of cryoprecipitation, such as Raynaud's phenomenon, may be present. Anaemia, at least partly caused by an increased plasma volume, is usually a significant problem and a bleeding tendency may result from macroglobulin interference with coagulation factors and platelet function. Neurological symptoms, dyspnoea and heart failure may be presenting symptoms. Moderate lymphadenopathy and enlargement of the liver and spleen are frequently seen.

Diagnosis

This is made by the finding of a monoclonal serum IgM together with bone marrow or lymph node infiltration with lymphoplasmacytoid cells. The ESR is raised and there may be a peripheral blood lymphocytosis.

Treatment

Specific

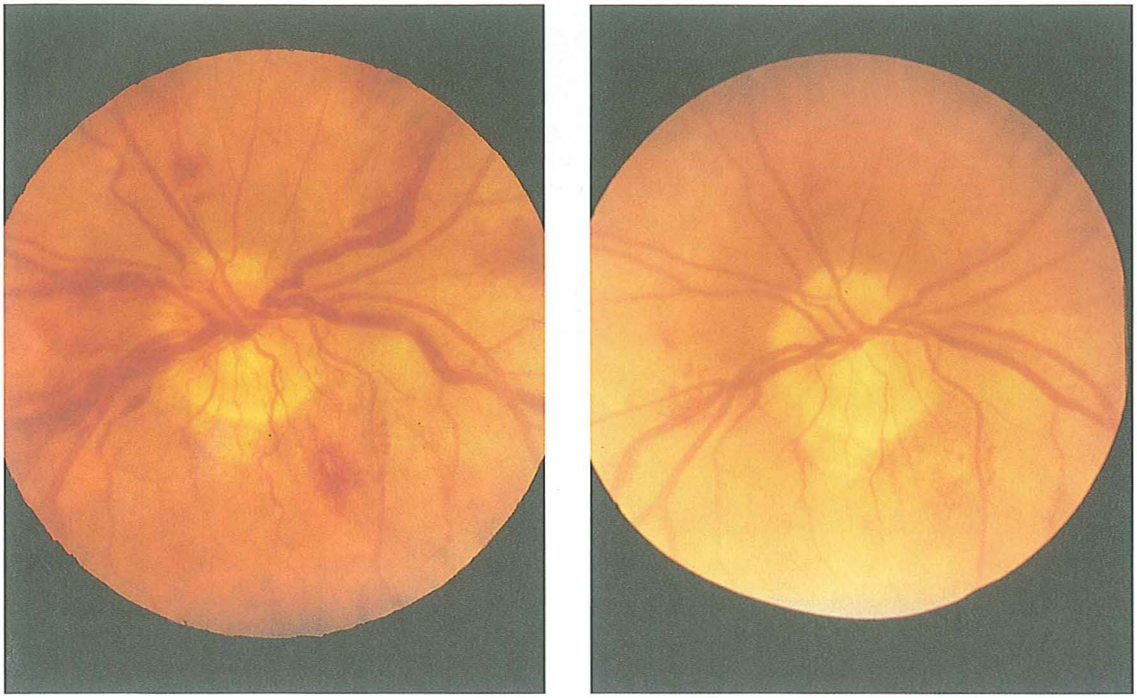
No therapy is needed for patients without symptoms, significant organomegaly or anaemia. Chlorambucil or cyclophosphamide have been the mainstay of therapy but fludarabine or 2-chlorodeoxyadenosine and rituximab are also useful for initial therapy or for relapsed cases. Combination chemotherapy as for non-Hodgkin's lymphoma is needed in late stages. SCT may be considered for advanced disease.

Support

Acute hyperviscosity syndrome is treated with repeated plasmapheresis. As IgM is mainly intravascular, plasmapheresis is more effective than with IgG or IgA paraproteins when much of the protein is extravascular and so rapidly replenishes the plasma compartment. Erythropoeitin or regular transfusions may be required for chronic anaemia.

Monoclonal gammopathy of undetermined significance

Transient or persistent paraproteins can occur in many other conditions as well as in multiple myeloma (Table 18.1). A serum paraprotein may be sometimes be detected without any evidence of myeloma or other underlying disease and is termed



(a) (b)

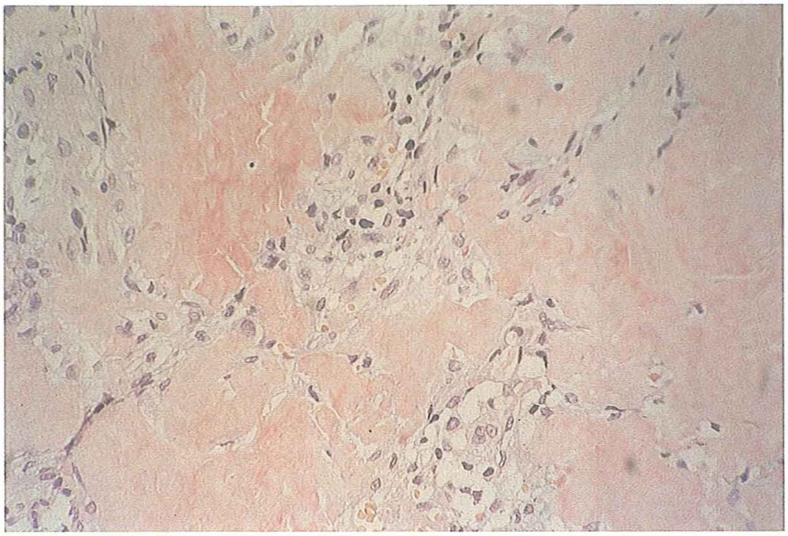
Fig. 18.9 Waldenström's macroglobulinaemia: hyperviscosity syndrome. **(a)** The retina before plasmapheresis shows distension of retinal vessels, particularly the veins which show bulging and constriction (the 'linked sausage' effect) and areas of haemorrhage; **(b)** following plasmapheresis the vessels have returned to normal and the areas of haemorrhage have cleared.

monoclonal gammopathy of undetermined significance (MGUS). It becomes increasingly common with age, being present in 1% of persons older than 50 years and 3% of those over 70 years. There are no bone lesions, no Bence-Jones proteinuria, and the proportion of plasma cells in the marrow is normal (<4%) or only slightly raised (<10%) (Table 18.2). The concentration of monoclonal immunoglobulin in serum is usually less than 20 g/L and other serum immunoglobulins are not depressed. Serum light chains (either κ or λ) are increased in one-third of patients; the greater the increase, the more the risk of transformation. DNA microarray patterns can distinguish myeloma from MGUS. No treatment is needed but patients with MGUS develop overt myeloma or lymphoma at a rate of approximately 1% each year and so are usually followed up regularly in the outpatient clinic.

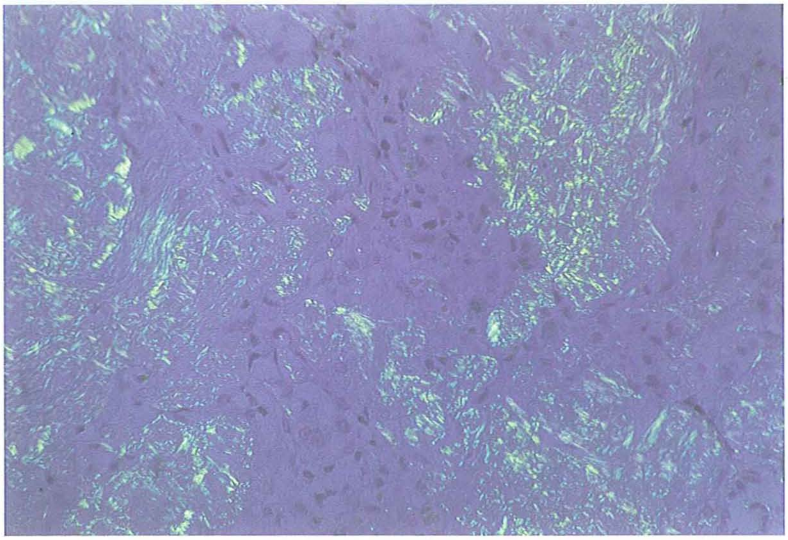
Amyloidosis

The amyloidoses are a heterogeneous group of disorders characterized by the extracellular deposition of protein in an abnormal fibrillar form. Amyloidosis may be hereditary or acquired and deposits may be focal, localized or systemic in distribution. The amyloid is made from different amyloid fibril precursor proteins in each type of disease. Except for intracerebral amyloid plaques, all amyloid deposits contain a non-fibrillary glycoprotein amyloid P which is derived from a normal serum precursor structurally related to CRP. The classic diagnostic histological test is red–green birefringence after staining with Congo red and viewing under polarized light (Fig. 18.10).

Amyloidosis is classified in Table 18.3.



(a)



(b)

Fig. 18.10 Amyloidosis: **(a)** Congo red staining; and **(b)** blue–green birefringence under polarized light.

Systemic AL amyloidosis

Systemic amyloid disease is caused by deposition of monoclonal light chains produced from a clonal plasma cell proliferation. The level of paraprotein may be very low and is not always detectable in serum or urine. The clinical features are caused by involvement of the heart, tongue (Fig. 18.11), peripheral nerves and kidneys (Fig. 18.12), and the patient may present with heart failure, macroglossia, peripheral neuropathy, carpal tunnel syndrome or renal failure. Treatment is with chemotherapy similar to that used in myeloma, possibly with autologous SCT, which may improve prognosis.

Hyperviscosity syndrome

The most common cause is polycythaemia (p. 233). Hyperviscosity may also occur in patients with myeloma or Waldenström's macroglobulinaemia

Table 18.3 Classification of amyloidosis: types, structure and organ involvement.

Type	Chemical nature	Organs involved
Systemic AL amyloidosis		
Associated with myeloma, Waldenström's macroglobulinaemia or MGUS May also occur on its own as primary amyloidosis (associated with an occult plasma cell proliferation) May also occur in localized form with local 'immunocyte' proliferation	Immunoglobulin light chains (AL)	Tongue Skin Heart Nerves Connective tissue Kidneys Liver Spleen
Reactive systemic AA amyloidosis		
Rheumatoid arthritis, tuberculosis, bronchiectasis, chronic osteomyelitis, inflammatory bowel disease Hodgkin's lymphoma, carcinomas, familial Mediterranean fever	Protein A (AA)	Liver Spleen Kidneys Bone marrow
Familial amyloidosis	e.g. Transthyretin abnormalities	Nerves Heart Eyes
Localized amyloidosis		
Central nervous system	β-amyloid protein	Alzheimer's disease
Endocrine	Peptic hormones	Endocrine tumours
Senile	Various	Heart, brain, joints, prostate, etc.

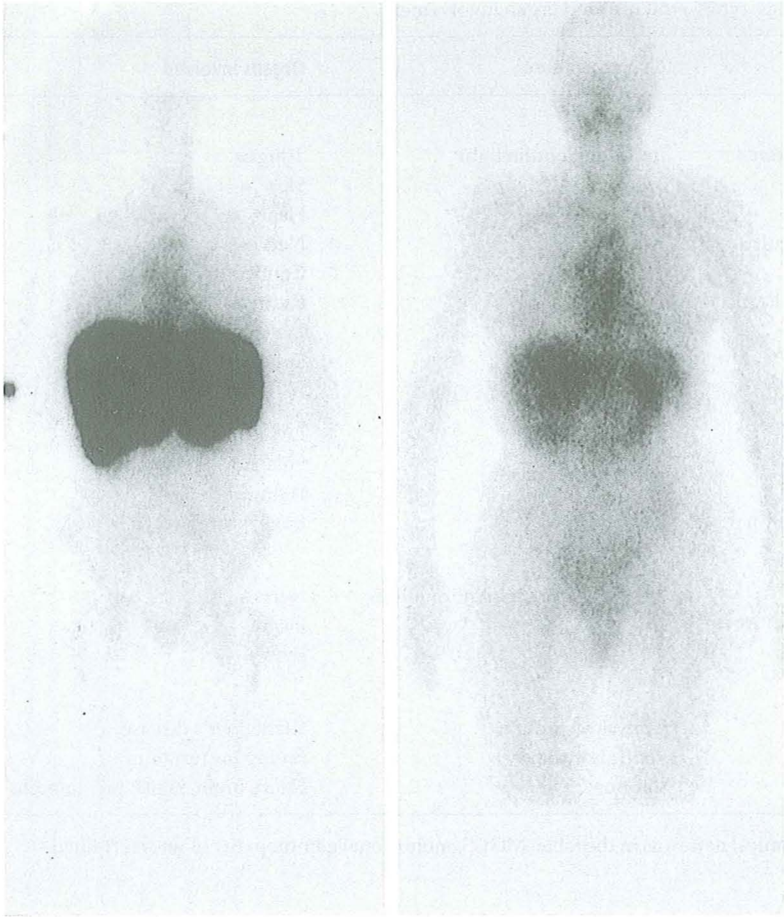
AA, AL, these are defined by their chemical nature as in the table; MGUS, monoclonal gammopathy of undetermined significance.

Fig. 18.11 Multiple myeloma: the tongue and lips are enlarged because of nodular and waxy deposits of amyloid.

or in patients with chronic or acute leukaemias associated with very high white cell counts. Rarely, haemophiliac patients with circulating inhibitors, being treated with massive doses of cryoprecipitate, have developed hyperviscosity because of the large volumes of fibrinogen infused.

The clinical features of the hyperviscosity syndrome include visual disturbances, lethargy, confusion, muscle weakness, nervous system symptoms and signs, and congestive heart failure. The retina may show a variety of changes: engorged veins, haemorrhages, exudates and a blurred disc (Fig. 18.9).

Emergency treatment varies with the cause: venesection or isovolaemic exchange with a plasma



(a) (b)

Fig. 18.12 Serial anterior whole body ^{123}I-labelled serum amyloid P component (SAP) scans of a 52-year-old woman who presented with renal failure resulting from systemic AL amyloidosis. **(a)** The initial scan demonstrates a large amyloid load with hepatic, splenic, renal and bone marrow deposits. The underlying plasma cell dyscrasia responded to high-dose melphalan followed by autologous stem cell rescue. **(b)** Follow-up SAP scintigraphy 3 years after chemotherapy showed greatly reduced uptake of tracer indicating substantial regression of her amyloid deposits. (Courtesy of Professor P.N. Hawkins, National Amyloidosis Centre, Royal Free Hospital, London)

substitute for red cells in a polycythaemic patient; plasmapheresis in myeloma, Waldenström's disease or hyperfibrinogenaemia; and leucopheresis or chemotherapy in leukaemias associated with high white counts. The long-term treatment depends on control of the primary disease with specific therapy.

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CHAPTER 19

19 Myeloproliferative disorders

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The term myeloproliferative disorders describes a group of conditions arising from marrow stem cells and characterized by clonal proliferation of one or more haemopoietic components in the bone marrow and, in many cases, the liver and spleen. Three disorders are included in this classification:

1 Polycythaemia rubra vera (PRV).
2 Essential thrombocythaemia (ET).
3 Myelofibrosis.

These disorders are closely related to each other. Indeed, transitional forms occur and in many patients an evolution from one entity into another occurs during the course of the disease (Fig. 19.1). A single acquired mutation of the cytoplasmic tyrosine kinase Janus-associated kinase 2 (JAK2) (Val617Phe) occurs (heterozygous or homozogous) in the marrow and blood of almost all patients with PRV and in approximately 50% of those with ET and myelofibrosis, showing the common aetiology of these three diseases (Fig. 19.2). The mutation occurs in a highly conserved region of the pseudokinase domain, which is believed to negatively regulate JAK2

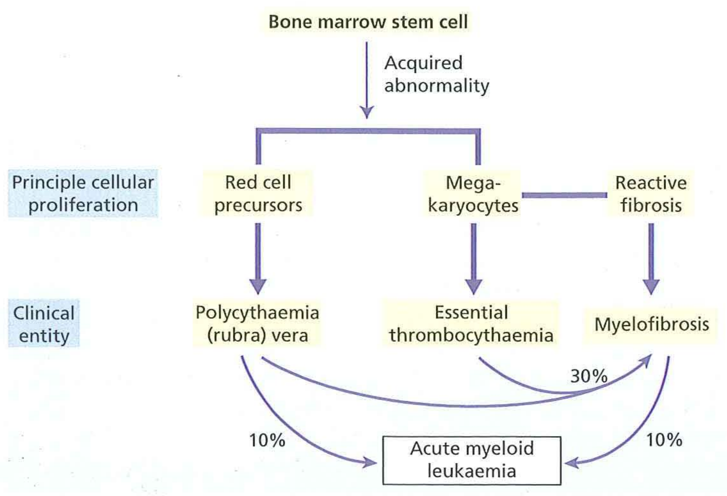


Fig. 19.1 Relationship between the three myeloproliferative diseases. They may all arise by somatic mutation in the pluripotential stem and progenitor cells. Many transitional cases occur showing features of two conditions and, in other cases, the disease transforms during its course from one of these diseases to another or to acute myeloid leukaemia. The three diseases, polycythaemia rubra vera, essential thrombocythaemia and myelofibrosis, are characterised by JAK2 mutation in a varying proportion of cases (see text).

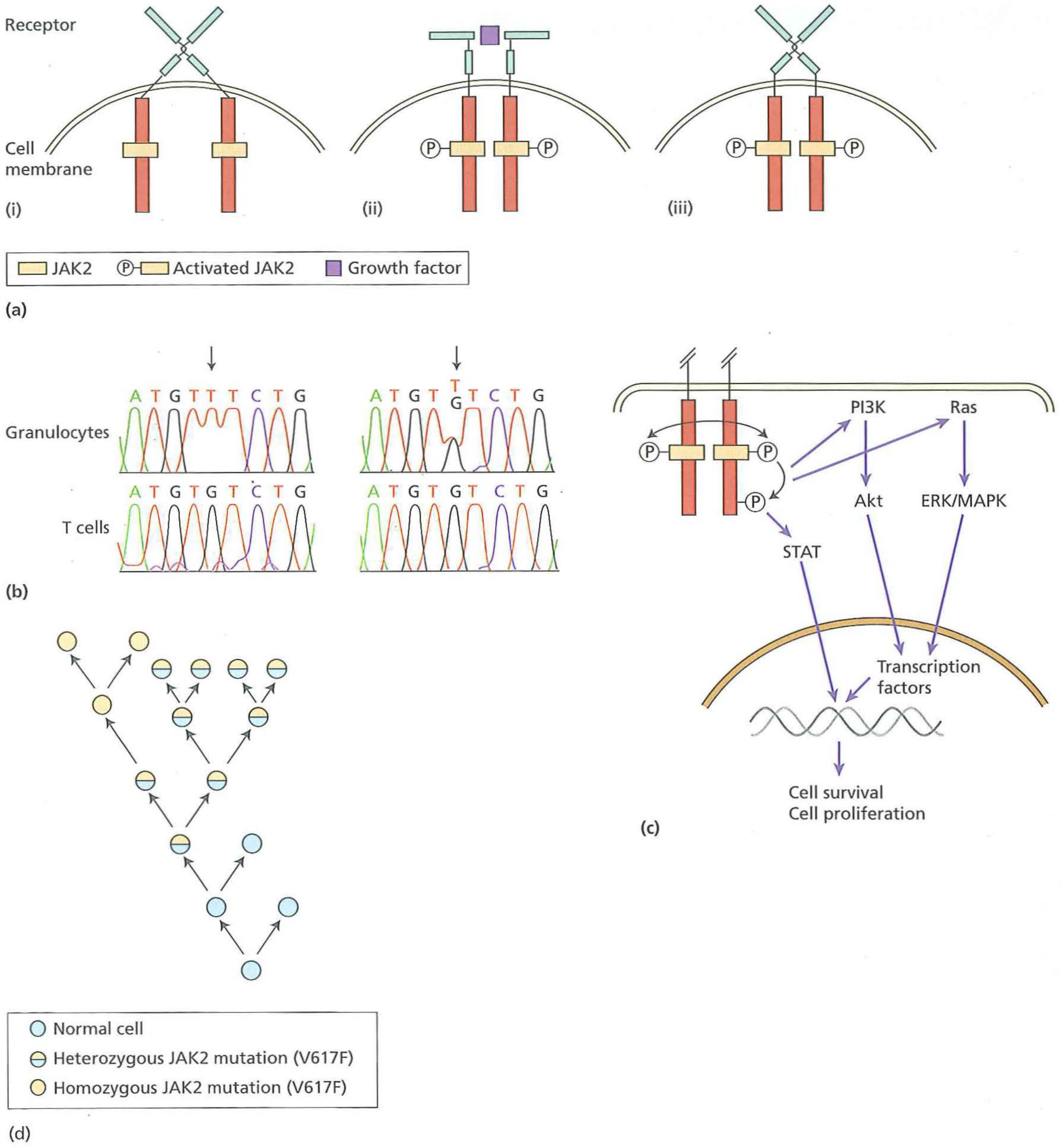


Fig. 19.2 The role of JAK2 mutation in the generation of myeloproliferative diseases. **(a)** (i) Most haemopoietic growth factor receptors do not have intrinsic kinase activity but associate with a protein kinase such as JAK2 in the cytoplasm. (ii) When the receptor binds a growth factor the cytoplasmic domains move closer together and the JAK2 molecules can activate each other by phosphorylation. (iii) The V617F JAK2 mutation allows the JAK protein to become activated even when no growth factor is bound. **(b)** DNA sequencing shows homozygous G → T mutation in JAK2 in granulocytes but not in T lymphocytes (left-hand panel) and heterozygous mutation in right-hand panel. (After Kralovic R. *et al.* (2005) *N Engl J Med* **352**,1779–90) **(c)** JAK2 activation leads to cell survival and proliferation through activation of three major pathways; the STAT transcription factors, the PI3K pathway acting through Akt and Ras activation which subsequently activate ERK and MAPK. The net result is production of a diverse range of proteins that promote cell survival and proliferation. **(d)** A model for the development of myeloproliferative disease following JAK2 mutation. The primary event appears to predispose to an acquired heterozygous mutation of JAK2 (V617F). This leads to a survival advantage. In some patients, a mitotic recombination event leads to a homozygous JAK2 mutation state.

Table 19.1 Radiodilution methods for measuring red cell and plasma volume.

	Normal	Primary or secondary polycythaemia	Relative polycythaemia
Total red cell volume (^{51}Cr)	Men 25–35 mL/kg Women 22–32 mL/kg	Increased	Normal
Total plasma volume (^{125}I-albumin)	40–50 mL/kg	Normal	Decreased

signalling. JAK2 plays a major role in normal myeloid development by transducing signals from diverse cytokines and growth factors including interleukin-3 (IL-3), erythropoietin, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and thrombopoietin (Fig. 1.8). Why the same mutation is associated with different myeloproliferative diseases is unclear. The exact cell in which the mutation arises, the genetic background of individual subjects and other factors may be relevant. Chronic myeloid leukaemia, which used to be classified as a myeloproliferative disorder but is now recognized as a separate entity, is discussed in Chapter 13.

Polycythaemia

Polycythaemia (erythrocytosis) is defined as an increase in the haemoglobin concentration above the upper limit of normal for the patient's age and sex.

Classification of polycythaemia

Polycythaemia is classified according to its pathophysiology but the major subdivision is into absolute polycythaemia, in which the red cell mass (volume) is raised, and relative or pseudopolycythaemia in which the red cell volume is normal but the plasma volume is reduced (Table 19.1). Absolute polycythaemia can then be subdivided into primary polycythaemia (PRV) or secondary polycythaemia (Table 19.2).

Table 19.2 Causes of polycythaemia.

Primary
Polycythaemia (rubra) vera
Familial (congenital) polycythaemia

Secondary
Caused by compensatory erythropoietin increase in:
- high altitudes
- pulmonary disease and alveolar hypoventilation (sleep apnoea)
- cardiovascular disease, especially congenital with cyanosis
- increased affinity haemoglobin (familial polycythaemia) (Chapter 19)
- heavy cigarette smoking

Caused by inappropriate erythropoietin increase in:
- renal diseases (e.g. hydronephrosis, vascular impairment, cysts, carcinoma)
- tumours such as uterine leiomyoma, hypernephroma, hepatocellular carcinoma, cerebellar haemangioblastoma

Relative
Stress or pseudopolycythaemia
Cigarette smoking
Dehydration: water deprivation, vomiting
Plasma loss: burns, enteropathy

Polycythaemia (rubra) vera

In PRV, the increase in red cell volume is caused by a clonal malignancy of a marrow stem cell. The disease results from somatic mutation of a single haemopoietic stem cell which gives its progeny a proliferative advantage. The *JAK2* mutation is present in haemopoietic cells in almost 100% of patients. Although the increase in red cells is the diagnostic finding, in many patients there is also an overproduction of granulocytes and platelets.

Chromosome abnormalities (e.g. deletions of 9p or 20q) are found in a minority of subjects.

Diagnosis

Making the diagnosis of PRV in a patient who

Table 19.3 Criteria for diagnosis of polycythaemia (rubra) vera.

Category A
Total red cell mass
male >35 mL/kg
female >32 mL/kg
Arterial oxygen saturation >92%
Splenomegaly
JAK2 mutation
Category B
Platelets $>400 \times 10^9$/L
White cells $>12 \times 10^9$/L
Increased NAP score
Raised serum vitamin B_{12} level

NAP, neutrophil alkaline phosphatase.

presents with polycythaemia can be difficult and the diagnostic criteria of the Polycythaemia Vera Study Group are very valuable (Table 19.3).

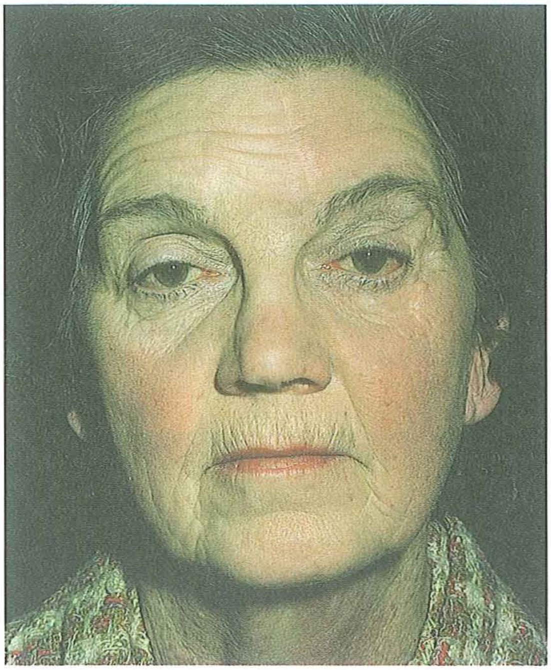


Fig. 19.3 Polycythaemia vera: facial plethora and conjunctival suffusion in a 63-year-old woman. Haemoglobin 18 g/dL; total red cell volume 45 mL/kg.

Clinical features

This is a disease of older subjects with an equal sex incidence. Clinical features are the result of hyperviscosity, hypervolaemia or hypermetabolism.

1 Headaches, dyspnoea, blurred vision and night sweats. Pruritus, characteristically after a hot bath, can be a severe problem.

2 Plethoric appearance: ruddy cyanosis (Fig. 19.3), conjunctival suffusion and retinal venous engorgement.

3 Splenomegaly in 75% of patients (Fig. 19.4a).

4 Haemorrhage (e.g. gastrointestinal, uterine, cerebral) or thrombosis either arterial (e.g. cardiac, cerebral, peripheral) or venous (e.g. deep or superficial leg veins, cerebral, portal or hepatic veins) are frequent.

5 Hypertension in one-third of patients.

6 Gout (as a result of raised uric acid production; Fig. 19.5a).

7 Peptic ulceration occurs in 5–10% of patients.

Laboratory findings

1 The haemoglobin, haematocrit and red cell count are increased. The total red cell volume (TRCV; Table 19.1) is increased.

2 A neutrophil leucocytosis is seen in over half of patients, and some have increased circulating basophils.

3 A raised platelet count is present in about half of patients.

4 The *JAK2* mutation is present in the bone marrow and peripheral blood granulocytes in nearly 100% of patients.

5 The neutrophil alkaline phosphatase (NAP) score is usually increased.

6 Increased serum vitamin B_{12} and vitamin B_{12}-binding capacity because of an increase in haptocorrin.

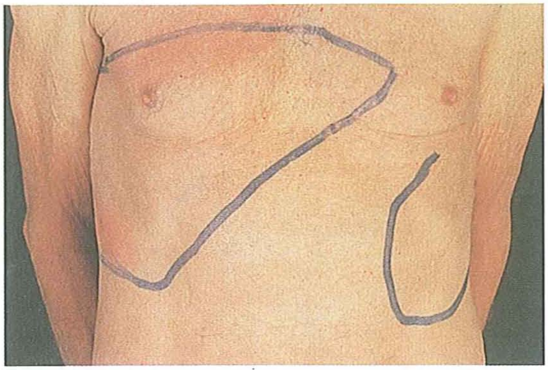
7 The bone marrow is hypercellular with prominent megakaryocytes, best assessed by a trephine biopsy (Fig. 19.6a).

8 Serum erythropoietin usually low.

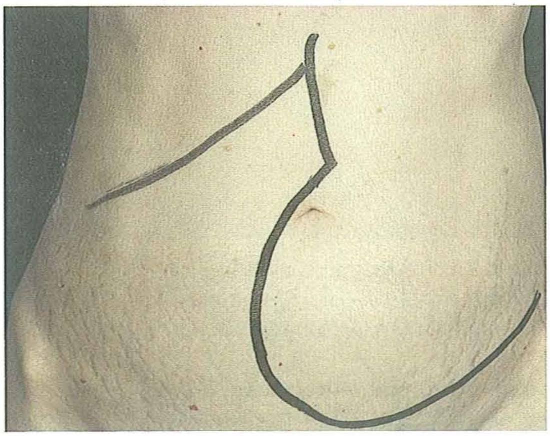
9 Expression of PRV-1 (a surface receptor) increased; reduced expression of Mpl (thrombopoietin receptor).

10 Blood viscosity is increased.

11 Plasma urate is often increased; the serum lactate dehydrogenase (LDH) is normal.



(a)



(b)

Fig. 19.4 Splenomegaly: enlarged spleens in male patients with **(a)** polycythaemia vera and **(b)** myelofibrosis.

12 Circulating erythroid progenitors (erythroid colony-forming unit, CFU_E, and erythroid burst-forming unit, BFU_E; p. 2) are increased compared to normal and grow *in vitro* independently of added erythropoietin (endogenous erythriod colonies).

Treatment

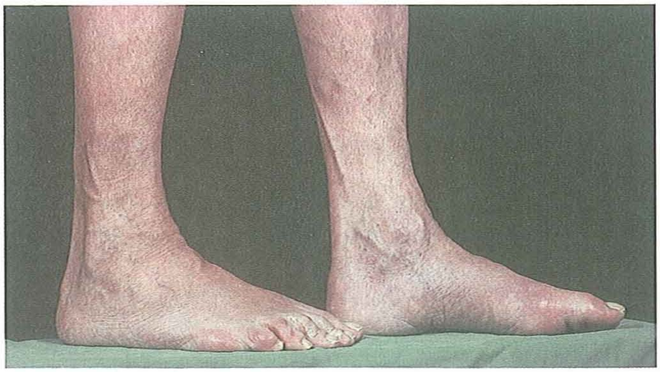
Treatment is aimed at maintaining a normal blood count. The haematocrit should be maintained at about 0.45 and the platelet count below 400×10^9/L.

Venesection

Venesection to reduce the haematocrit to less than 0.45 is particularly useful when a rapid reduction of red cell volume is required (e.g. at the start of therapy). It is especially indicated in younger patients and those with mild disease. The resulting iron deficiency may limit erythropoiesis. Unfortunately, venesection does not control the platelet count.

Cytotoxic myelosuppression

This is considered if there is poor tolerance of venesection, symptomatic or progressive splenomegaly, thrombocytosis, weight loss or night sweats. Daily hydroxycarbamide (hydroxyurea) is valuable in controlling the blood count and may need to be continued for many years (Fig. 19.7). Side-effects of hydroxyurea include myelosuppression, nausea



(a)

(b)

Fig. 19.5 **(a)** The feet of a 72-year-old man with polycythaemia rubra vera. There is inflammation of the right metatarsophalangeal and other joints caused by uric acid deposits. **(b)** Gangrene of the left fourth toe in essential thrombocythaemia.

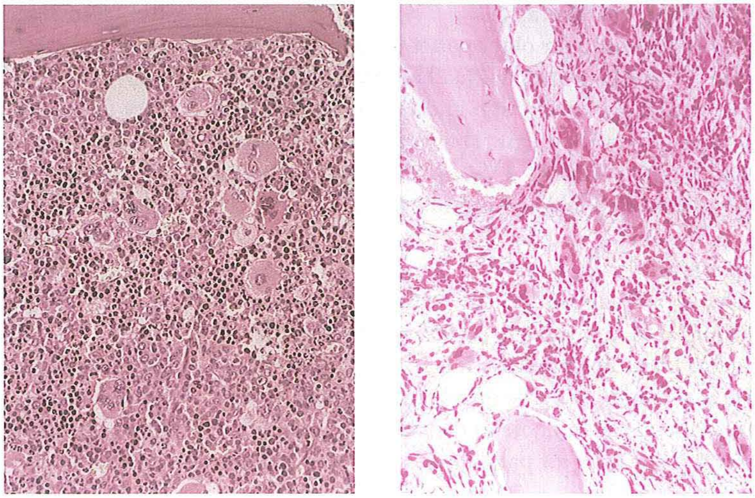


Fig. 19.6 Iliac crest trephine biopsies. **(a)** Polycythaemia vera: fat spaces are almost completely replaced by hyperplastic haemopoietic tissue. All haemopoietic cell lines are increased with megakaryocytes particularly prominent. **(b)** Myelofibrosis: normal marrow architecture is lost and haemopoietic cells are surrounded by increased fibrous tissue and intercellular substance.

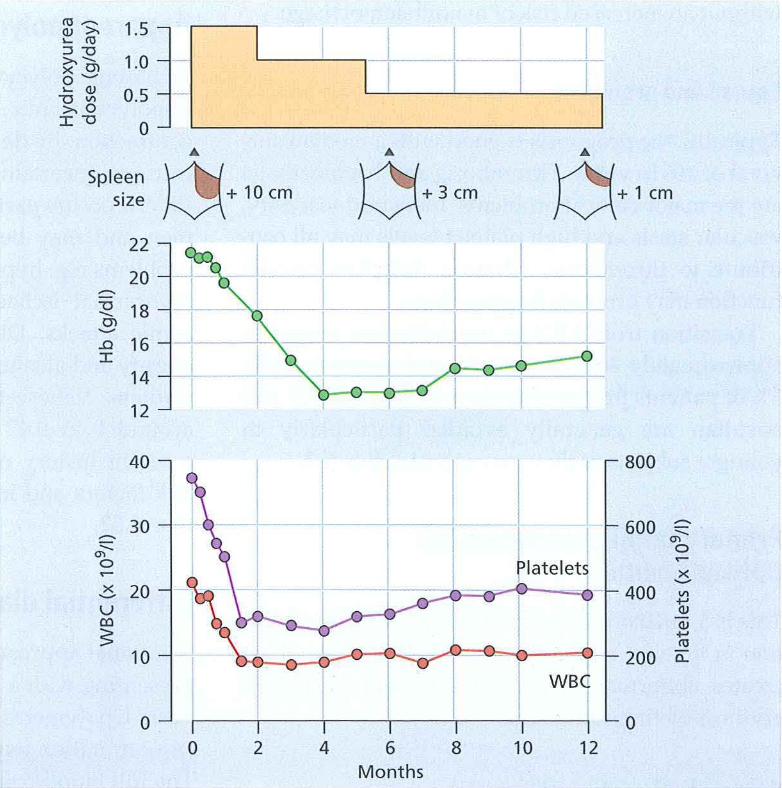


Fig. 19.7 Haematological response to therapy with hydroxyurea in polycythaemia vera. Hb, haemoglobin; WBC, white blood cells.

and skin toxicity. Busulfan, which can be used intermittently, is sometimes used in older patients. Pipobroman is similar to alkyating agents and is used in Europe but not in the UK. The concern with cytotoxic drugs, especially busulfan, is that they may be associated with an increased rate of progression to leukaemia. This risk is low and there may be no increased risk with hydroxyurea.

Phosphorus-32 therapy

This is only used for older patients with severe disease. ^{32}P is a β-emitter, with a half-life of 14.3 days. It is concentrated in bone and is a most effective myelosuppressive agent. The usual remission time after a single dose is 2 years. Concern about late development of leukaemia limits its use.

Interferon

α-Interferon subcutaneously suppresses excess proliferation in the marrow and has produced good haematological responses. It is less convenient than the oral agents and side-effects are frequent. It may be particularly valuable in controlling itching. It is the first-line drug for patients less than 40 years old.

Aspirin

Low-dose aspirin reduces thrombotic complications without an increased risk of major haemorrhage.

Course and prognosis

Typically, the prognosis is good with a median survival of 10–16 years. Thrombosis and haemorrhage are the major clinical problems. Increased viscosity, vascular stasis and high platelet levels may all contribute to thrombosis, whereas defective platelet function may promote haemorrhage.

Transition from PRV to myelofibrosis occurs in approximately 30% of patients and approximately 5% of patients progress to acute leukaemia. ^{32}P and busulfan are generally avoided particularly in younger subjects as they may increase this risk.

Primary familial (congenital) polycythaemia

This is a rare condition. In one type there is a mutation in the von Hippel–Lindau protein gene which creates abnormal oxygen sensing with increased erythropoietin production.

Secondary polycythaemia

The causes of secondary polycythaemia are listed in Table 19.2. Hypoxia caused by chronic obstructive airways disease is one of the most frequent, and measurement of arterial oxygen saturation is a valuable test. Renal and tumour causes of inappropriate erythropoietin secretion are rare. Patients with a high-affinity haemoglobin often have a family history of polycythaemia and present at a young age. Those with cyanotic congenital heart disease should be managed in conjunction with a congenital heart disease unit. Treatment of obesity, hypertension and discontinuation of smoking may lower the haematocrit. These patients are treated by venesection for symptoms such as dizziness, dyspnoea or angina if a raised haematocrit is a contributory factor; also if there has been a previous thrombotic episode. Patients with polycythaemia secondary to hypoxia and pulmonary disease who have symptoms of hyperviscosity or haematocrit of more than 0.56 should have their haematocrit reduced by venesection.

Apparent polycythaemia

Apparent polycythaemia, also known as pseudopolycythaemia, is the result of plasma volume contraction. By definition, the TRCV is normal. The cause is uncertain but it is far more common than PRV. It occurs particularly in young or middle-aged men and may be associated with cardiovascular problems e.g. hypertension (Gaisböck's syndrome) myocardial ischaemia or cerebral transient ischaemic attacks. Diuretic therapy, heavy smoking, obesity and alcohol consumption are frequent associations. Venesection to maintain a haematocrit around 0.45–0.47 is recommended in those with a recent history of thrombosis or with additional risk factors and in all with a haematocrit of more than 0.52.

Differential diagnosis of polycythaemia

A rational approach is needed to evaluate a patient presenting with a high haemoglobin. If PRV is suspected, polymerase chain reaction (PCR) test for the *JAK2* mutation will confirm this in almost all cases. The full blood count, the NAP score, bone marrow aspirate trephine biopsy and ultrasound of the abdomen to assess spleen size and to detect renal abnormalities are the most useful additional tests. Studies with ^{51}Cr-labelled red cells to measure TRCV may be needed if *JAK2* is negative. If absolute

polycythaemia is present look for lung or cardiac disease, check arterial Po_2 and consider a check of the oxygen dissociation curve and haemoglobin (Hb) electrophoresis. Finally, look for erythropoietin secreting tumours by ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI). The serum erythropoietin level is also useful in screening for tumours.

Essential thrombocythaemia

In this condition there is a sustained increase in platelet count, because of megakaryocyte proliferation and overproduction of platelets. The haematocrit is normal and the Philadelphia chromosome or *BCR-ABL* rearrangement are absent. The bone marrow shows no collagen fibrosis. A persisting platelet count of $>400 \times 10^9$/L is the central diagnostic feature but other causes of a raised platelet count (particularly iron-deficiency, inflammatory or malignant disorder and myelodysplasia) need to be fully excluded before the diagnosis can be made. Half of patients show the *JAK2* (Val617Phe) mutation and these cases tend to resemble more closely polycythaemia vera with higher haemoglobin and white cell counts and splenomegaly than *JAK2* negative cases. There is endogenous megakaryocyte and possibly erythroid colony growth independent of thrombopoietin or erythropoietin. Rare primary familial cases in children have been associated with mutations on the genes for thrombopoietin or its receptor *c*-Mpl.

Clinical and laboratory findings

The dominant clinical features are thrombosis and haemorrhage. Many cases are symptomless and diagnosed on routine blood counts. Thrombosis may occur in the venous or arterial systems (Fig. 19.5b) whereas haemorrhage, as a result of abnormal platelet function, may cause either chronic or acute bleeding. A characteristic symptom is erythromelalgia, a burning sensation felt in the hands or feet and promptly relieved by aspirin. Up to 40% of patients will have palpable splenomegaly whereas in others there may be splenic atrophy because of infarction. Abnormal large platelets and megakaryocyte fragments may be seen on the blood film (Fig. 19.8). The bone marrow is similar to that in PRV but an excess of abnormal megakaryocytes is typical. Cytogenetics and molecular analysis for the *BCR-ABL* fusion gene are analysed to exclude chronic myeloid leukaemia. The condition must be distinguished from other causes of a raised platelet count (Table 19.4). Platelet function tests (p. 277) are consistently abnormal, failure of aggregation with adrenaline being particularly characteristic.

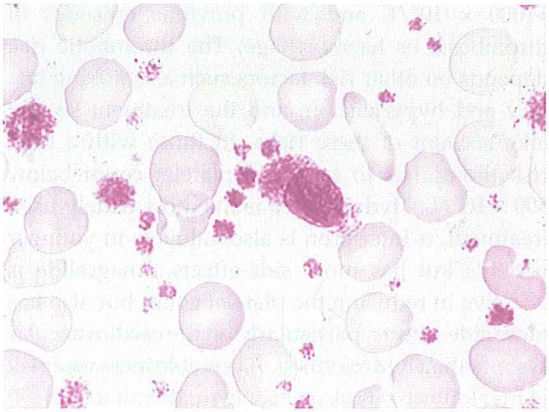


Fig. 19.8 Peripheral blood film in essential thrombocythaemia showing increased numbers of platelets and a nucleated megakaryocytic fragment.

Table 19.4 Causes of a raised platelet count.

Reactive
Haemorrhage, trauma, postoperative
Chronic iron deficiency
Malignancy
Chronic infections
Connective tissue diseases (e.g. rheumatoid arthritis)
Post-splenectomy
Endogenous
Essential thrombocythaemia (*JAK2* mutation + or –)
In some cases of polycythaemia vera, myelofibrosis and chronic myeloid leukaemia

Treatment

The principle is to control the platelet count so as to reduce the risk of thrombosis which is the major clinical problem. The patients most at risk are those over 60 years old, with a platelet count

>1000 × 10^9/L and with previous episodes of thrombosis or haemorrhage. The thrombotic risk depends on other risk factors such as smoking history and hypertension, and the treatment should take account of these risks. In those with a high risk the aim is to keep the platelet count below 600 × 10^9/L. Hydroxyurea is the most widely used treatment. α-Interferon is also valuable in younger patients but has more side-effects. Anagrelide is effective in reducing the platelet count but also has more side-effects, particularly on the cardiovascular system, than hydroxyurea. A possible increased risk of myelofibrosis is also of concern. Busulfan and ^{32}P were used but are not now favoured because of possible long-term complications. Platelet pheresis may be helpful in short-term management. Aspirin is commonly used to reduce thrombotic risk and in patients younger than 60 years with no previous thrombosis or haemorrhage, and platelets <1000 × 10^9/L, it may be the treatment of choice.

Course

Often the disease is stationary for 10–20 years or more. The disease may transform after a number of years to myelofibrosis; the risk of transformation to acute leukaemia is relatively low (<5%). Patients have been stratified as low risk if less than 40 years old, with no previous thrombosis, hypertension or diabetes and platelets <1000–1500 × 10^9/L and are treated with aspirin alone; intermediate risk if less than 60 years with no prior thrombosis, hypertension or diabetes and platelets <1000–1500 × 10^9/L; or high risk if over 60 years or with prior thrombosis or haemorrhage, hypertension or diabetes, platelet count >1000 × 10^9/L. High-risk patients receive hydroxyurea and aspirin. For intermediate risk, trials of aspirin versus hydroxyurea and aspirin are in progress.

Myelofibrosis

Confusingly, the condition has many names: idiopathic myelofibrosis; myelosclerosis; agnogenic myeloid metaplasia; or myelofibrosis with myeloid metaplasia (MMM).

The predominant feature of myelofibrosis is a progressive generalized reactive fibrosis of the bone marrow in association with the development of haemopoiesis in the spleen and liver (known as myeloid metaplasia). Clinically this leads to anaemia and massive hepatosplenomegaly. In some patients there is osteosclerosis. Myelofibrosis is a clonal stem cell disease. The fibrosis of the bone marrow is secondary to hyperplasia of abnormal megakaryocytes. It is thought that fibroblasts are stimulated by platelet-derived growth factor and other cytokines secreted by megakaryocytes and platelets. The *JAK2* mutation occurs in approximately 50% of patients. The absence of the mutation in many patients shows genetic heterogeneity in myelofibrosis as in ET. Non-specific cytogenetic abnormalities may be found in approximately half the patients.

One-third or more of the patients have a previous history of PRV and some patients present with clinical and laboratory features of both disorders.

Clinical features

1 An insidious onset in older people is usual with symptoms of anaemia.
2 Symptoms resulting from massive splenomegaly (e.g. abdominal discomfort, pain or indigestion) are frequent; splenomegaly is the main physical finding (Fig. 19.4b).
3 Hypermetabolic symptoms such as loss of weight, anorexia, fever and night sweats are common.
4 Bleeding problems, bone pain or gout occur in a minority of patients.

Myelofibrosis and chronic myeloid leukaemia are responsible for most cases of massive (>20 cm) splenic enlargement in the UK and North America (see Table 9.1).

Laboratory findings

1 Anaemia is usual but a normal or increased haemoglobin level may be found in some patients.
2 The white cell and platelet counts are frequently high at the time of presentation. Later in the disease leucopenia and thrombocytopenia are common.
3 A leucoerythroblastic blood film is found. The red cells show characteristic 'tear-drop' poikilocytes (Fig. 19.9).
4 Bone marrow is usually unobtainable by aspiration. Trephine biopsy (Fig. 19.6b) shows a fibrotic

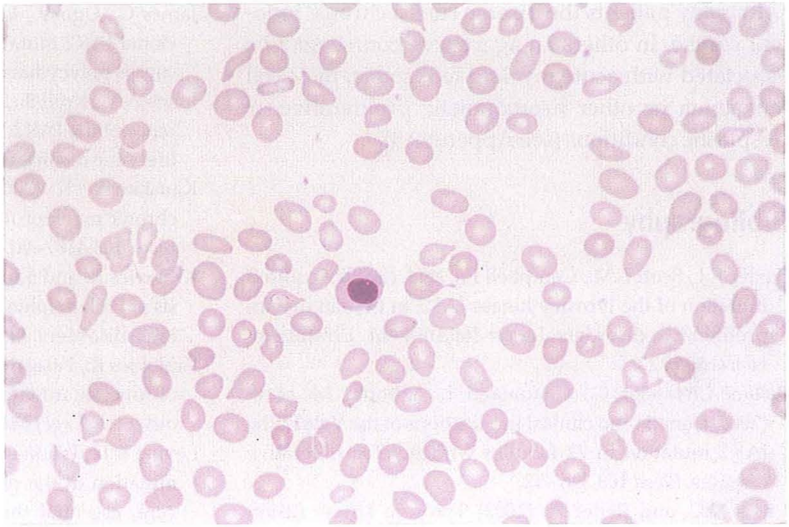


Fig. 19.9 Peripheral blood film in myelofibrosis. Leucoerythroblastic change with 'tear-drop' cells and an erythroblast.

hypercellular marrow. Increased megakaryocytes are frequently seen. In 10% of cases there is increased bone formation with increased bone density on X-ray.

5 *JAK2* kinase is mutated in approximately 50% of cases.

6 NAP score is usually increased.

7 High serum urate and LDH levels reflect the increased but largely ineffective turnover of haemopoietic cells.

8 Transformation to acute myeloid leukaemia occurs in 10–20% of patients.

Treatment

This is usually palliative and aimed at reducing the effects of anaemia and splenomegaly. Blood transfusions and regular folic acid therapy are used in severely anaemic patients. Hydroxyurea may help to reduce splenomegaly and hypermetabolic symptoms. Trials of thalidomide, cyclophosphamide or etanercept (anti-TNFα) are in progress. Danazol, an androgen derivative may improve anaemia in approximately 30% of patients. Erythropoietin may also be tried. Splenectomy is considered for patients with severe symptomatic splenomegaly—mechanical discomfort, thrombocytopenia, portal hypertension, excessive transfusion requirements or hypermetabolic symptoms. Splenic irradiation is an alternative but usually provides relief only for 3–6 months. Allopurinol is indicated in virtually all patients to prevent gout and urate nephropathy from hyperuricaemia. Allogeneic stem cell transplantation may be curative for young patients.

The median survival is approximately 3.5 years and causes of death include heart failure, infection and leukaemic transformation. A haemoglobin level of less than 10 g/dL, a white cell count of less than 4 or greater than 30×10^9/L and the presence of abnormal chromosomes are associated with a worse prognosis.

Systemic mastocytosis

Mast cells are derived from haemopoietic stem cells. Mature cells survive for months or years in vascular tissues and most organs. Systemic mastocytosis is a clonal myeloproliferative disorder involving usually the bone marrow, heart, spleen, lymph nodes and skin.

The somatic *c*-kit mutation Asp816Val is detected in the majority of patients and may be partly responsible for autonomous growth and enhanced survival of the neoplastic mast cells. In many patients this mutation is also detected in other haemopoietic cells.

Symptoms are related to histamine and prostaglandin release and include flushing, pruritus, abdominal pain and bronchospasm. The skin usually shows urticaria pigmentosa.

In many patients the disease runs a chronic indolent course. In others an aggressive course may be associated with acute myeloid leukaemia, mast cell leukaemia or other haemopoietic proliferative or dysplastic conditions (see Appendix 3).

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CHAPTER 20

20 Aplastic anaemia and bone marrow failure

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Pancytopenia

Pancytopenia describes a reduction in the blood count of all the major cell lines—red cells, white cells and platelets. It has several causes (Table 20.1) which can be broadly divided into decreased bone marrow production or increased peripheral destruction.

Aplastic anaemia

Aplastic (hypoplastic) anaemia is defined as pancytopenia resulting from aplasia of the bone marrow. It is classified into primary (congenital or acquired) or secondary types (Table 20.2).

Pathogenesis

The underlying defect in all cases appears to be a substantial reduction in the number of haemopoietic pluripotential stem cells, and a fault in the remaining stem cells or an immune reaction against them, which makes them unable to divide and differentiate sufficiently to populate the bone marrow (Fig. 20.1). A primary fault in the marrow microenvironment has also been suggested but the success of stem cell transplantation (SCT) shows this can only be a rare cause because normal donor stem cells are usually able to thrive in the recipient's marrow cavity.

Table 20.1 Causes of pancytopenia.

Decreased bone marrow function
Aplasia
Acute leukaemia, myelodysplasia, myeloma
Infiltration with lymphoma, solid tumours, tuberculosis
Megaloblastic anaemia
Paroxysmal nocturnal haemoglobinuria
Myelofibrosis
Haemophagocytic syndrome
Increased peripheral destruction
Splenomegaly

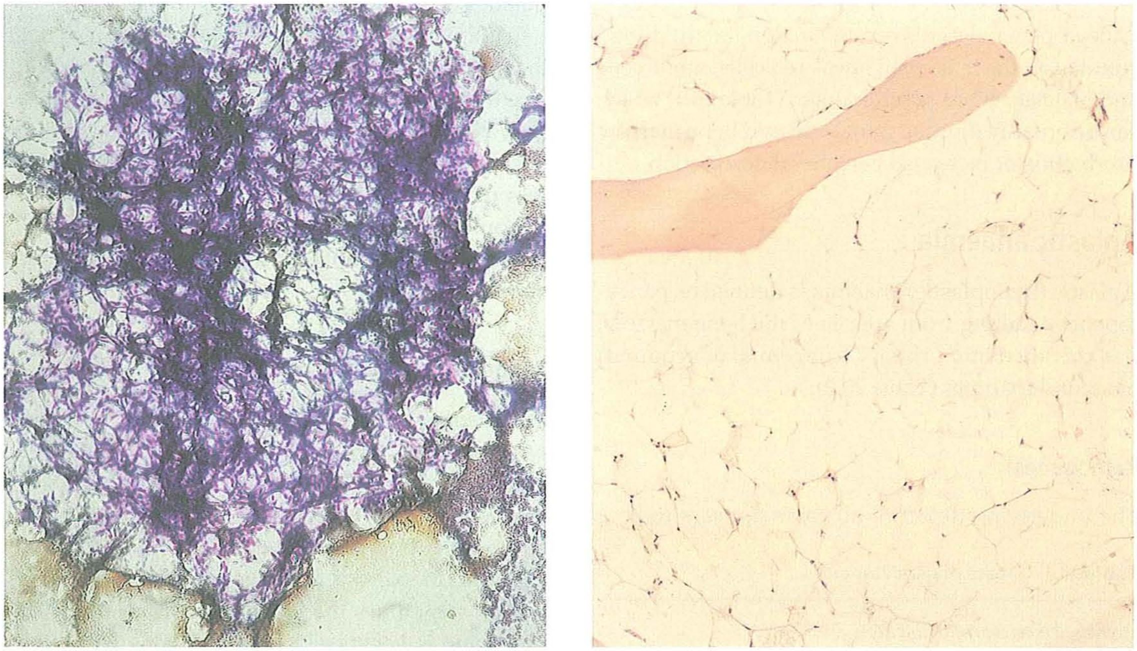
Congenital

The Fanconi type has an autosomal recessive pattern of inheritance and is often associated with growth retardation and congenital defects of the skeleton (e.g. microcephaly, absent radii or thumbs), of the renal tract (e.g. pelvic or horseshoe kidney) (Fig. 20.2) or skin (areas of hyper- and hypopigmentation); sometimes there is mental retardation. The syndrome is genetically heterogeneous with eight different complementation groups termed A, B, C, D1, D2, E, F and G. The *FAND1* gene is identical to *BRCA2*, the breast cancer susceptibility gene. The encoded proteins cooperate in a common cellular pathway involved in DNA repair. Cells from Fanconi's anaemia (FA) patients show an abnormally high frequency of spontaneous chromosomal breakage and the diagnostic test is elevated breakage after incubation of peripheral blood lymphocytes

Table 20.2 Causes of aplastic anaemia.

Primary	Secondary
Congenital (Fanconi and non-Fanconi types) Idiopathic acquired	**Ionizing radiation**: accidental exposure (radiotherapy, radioactive isotopes, nuclear power stations) **Chemicals**: benzene, organophosphates and other organic solvents, DDT and other pesticides, organochlorines, recreational drugs (ecstasy) **Drugs** Those that regularly cause marrow depression (e.g. busulfan, cyclophosphamide, anthracyclines, nitrosoureas) Those that occasionally or rarely cause marrow depression (e.g. chloramphenicol, sulphonamides, gold, anti-inflammatory, antithyroid, psychotrophic, anticonvulsant/antidepressant drugs) **Viruses**: viral hepatitis (non-A, non-B, non-C, non-G in most cases), EBV

EBV, Epstein–Barr virus.

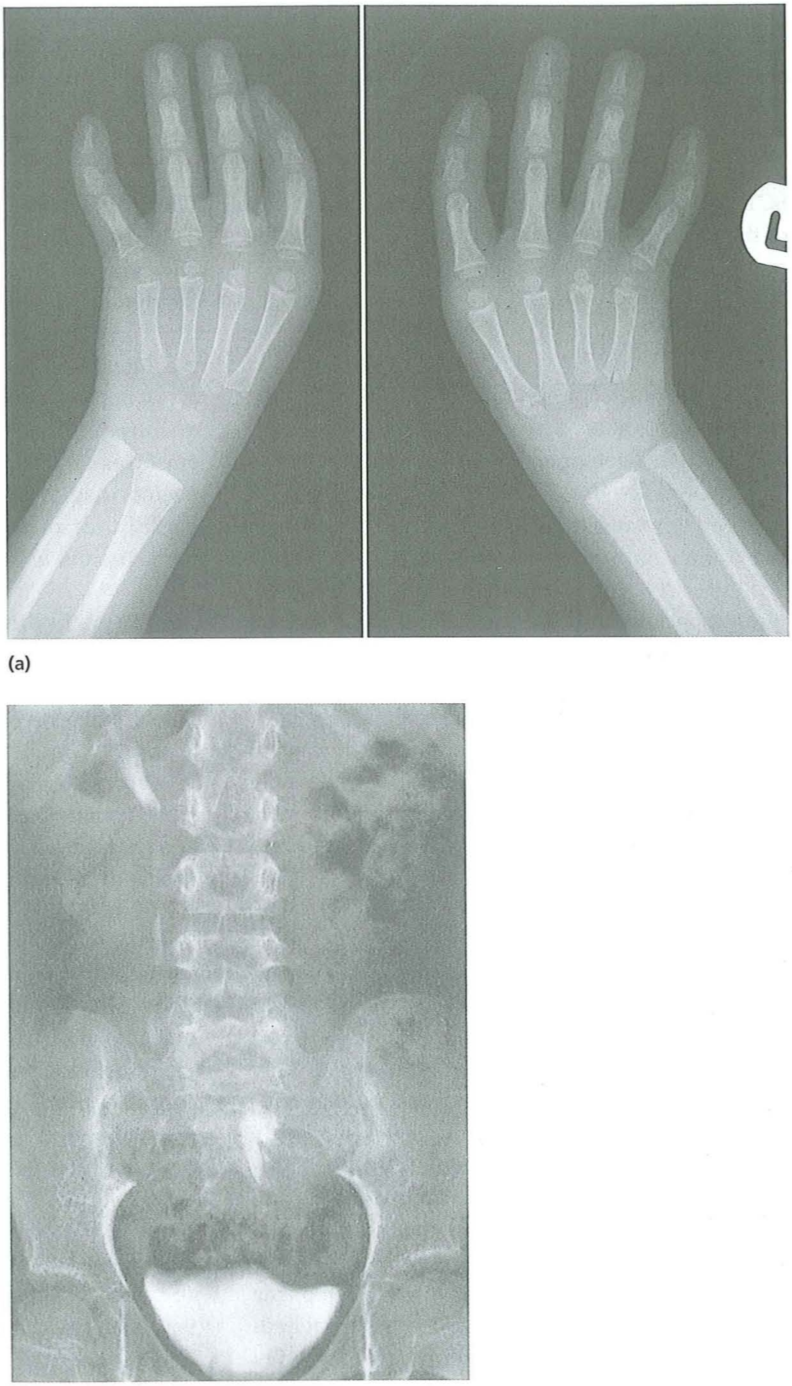


(a) (b)

Fig. 20.1 Aplastic anaemia: low power views of bone marrow show severe reduction of haemopoietic cells with an increase in fat spaces. **(a)** Aspirated fragment. **(b)** Trephine biopsy.

with the DNA cross-linking agent diepoxybutane (DEB test). Dyskeratosis congenita is a rare sex-linked disorder with nail and skin atrophy and is associated with mutations in the *DKC1* (dyskerin) or *TERC* (telomerase reverse transcriptase RNA template) genes which are both involved in the maintenance of telomere length.

The usual age of presentation of FA is 5–10 years.



(a)

(b)

Fig. 20.2 **(a)** X-rays showing absent thumbs in a patient with Fanconi's anaemia (FA). **(b)** Intravenous pyelogram in a patient with FA showing a normal right kidney but a left kidney abnormally placed in the pelvis.

Approximately 10% of cases develop acute myeloid leukaemia. Treatment is usually with androgens and/or SCT. The blood count usually improves with androgens but side-effects, especially in children, are distressing (virilization and liver abnormalities); remission rarely lasts more than 2 years. SCT may cure the patient. Because of the sensitivity of the patient's cells to DNA damage, conditioning regimes are mild.

Idiopathic acquired

This is the most common type of aplastic anaemia, accounting for at least two-thirds of acquired cases. In most cases haemopoetic tissue is the target of an immune process dominated by oligoclonal expression of cytotoxic T cells which secrete γ-interferon and tumour necrosis factor. In approximately one-third of cases short telomeres are found in leucocytes, especially in those with a prolonged clinical course. Mutations in the telomere repair complex have been described but their relevance is unclear. The favourable responses to antilymphocyte globulin (ALG) and ciclosporin support the concept that autoimmune T-cell mediated damage, possibly against functionally and structurally altered stem cells, is important.

Secondary

This is often caused by direct damage to the haemopoietic marrow by radiation or cytotoxic drugs. The antimetabolite drugs (e.g. methotrexate) and mitotic inhibitors (e.g. daunorubicin) cause only temporary aplasia but the alkylating agents, particularly busulfan, may cause chronic aplasia closely resembling the chronic idiopathic disease. Some individuals develop aplastic anaemia as a rare idiosyncratic side-effect of drugs such as chloramphenicol or gold which are not known to be cytotoxic (Table 20.2). They may also develop the disease during or within a few months of viral hepatitis (rarely hepatitis A, B or C, but more frequently non-A, non-B, non-C). Because the incidence of marrow toxicity is particularly high for chloramphenicol, this drug should be reserved for treatment of those infections that are life-threatening and for which it is the optimum antibiotic (e.g. typhoid). Chemicals such as benzene may be implicated and rarely aplastic anaemia may be the presenting feature of acute lymphoblastic or myeloid leukaemia, especially in childhood. Myelodysplasia (Chapter 14) may also present with a hypoplastic marrow.

Clinical features

The onset is at any age with a peak incidence around 30 years and a slight male predominance; it can be insidious or acute with symptoms and signs resulting from anaemia, neutropenia or thrombocytopenia. Infections, particularly of the mouth and throat, are common and generalized infections are frequently life-threatening; bruising, bleeding gums, epistaxes and menorrhagia are the most frequent haemorrhagic manifestations and the usual presenting features, often with symptoms of anaemia. The lymph nodes, liver and spleen are not enlarged.

Laboratory findings

1 Anaemia is normochromic, normocytic or macrocytic (mean cell volume (MCV) often 95–110 fL). The reticulocyte count is usually extremely low in relation to the degree of anaemia.

2 Leucopenia. There is a selective fall in granulocytes, usually but not always to below 1.5×10^9/L. In severe cases, the lymphocyte count is also low. The neutrophils appear normal.

3 Thrombocytopenia is always present and, in severe cases, is less than 20×10^9/L.

4 There are no abnormal cells in the peripheral blood.

5 The bone marrow shows hypoplasia, with loss of haemopoietic tissue and replacement by fat which comprises over 75% of the marrow. Trephine biopsy is essential and may show patchy cellular areas in a hypocellular background (Fig. 20.1b). The main cells present are lymphocytes and plasma cells; megakaryocytes in particular are severely reduced or absent.

Severe cases show neutrophils $<0.5 \times 10^9$/L (very severe $<0.2 \times 10^9$/L), platelets $<20 \times 10^9$/L, reticulocytes $<20 \times 10^9$/L and marrow cellularity <25%.

Diagnosis

The disease must be distinguished from other causes of pancytopenia (Table 20.1) and this is not

usually difficult provided an adequate bone marrow sample is obtained. Cytogenetic analysis should be performed. Paroxysmal nocturnal haemoglobinuria (PNH) must be excluded by flow-cytometry testing of red cells for CD55 and CD59. In older patients, hypoplastic myelodysplasia may show similar appearances. Qualitative abnormalities of the cells and clonal cytogenetic changes suggest myelodysplasia rather than aplastic anaemia. Some patients diagnosed as having aplastic anaemia develop PNH, myelodysplasia or acute myeloid leukaemia in subsequent years. This may occur even in patients who have responded well to immunosuppressive therapy.

Treatment

General

The cause, if known, is removed (e.g. radiation or drug therapy is discontinued). Initial management consists largely of supportive care with blood transfusions, platelet concentrates, and treatment and prevention of infection. All blood products should be filtered to reduce the risk of alloimmunization and irradiated to prevent grafting of live donor lymphocytes. In severely thrombocytopenic (platelet count $<10 \times 10^9$/L) and neutropenic (neutrophils $<0.5 \times 10^9$/L) patients, management is similar to the supportive care of patients receiving intensive chemotherapy with reverse barrier isolation. An antifibrinolytic agent (e.g. tranexamic acid) may be used in patients with severe prolonged thrombocytopenia. Oral antifungal agents and oral antibiotics are used prophylactically in some units to reduce the incidence of infection.

Specific

This must be tailored to the severity of the illness as well as the age of the patient and potential sibling stem cell donors. Severity is assessed by the reticulocyte, neutrophil and platelet counts and degree of marrow hypoplasia. Severe cases have a high mortality in the first 6–12 months unless they respond to specific therapy. Less severe cases may have an acute transient course or a chronic course with ultimate recovery, although the platelet count often remains subnormal for many years. Relapses, sometimes severe and occasionally fatal, may also occur and rarely the disease transforms into myelodysplasia, acute leukaemia or PNH (Chapter 5).

The following 'specific' treatments are used with varying success.

1 *Antilymphocyte or antithymocyte globulin* (ALG or ATG) This is prepared in animals (e.g. horse or rabbit) and is of benefit in approximately 50–60% of acquired cases. It is usually given with corticosteroids which also reduce the side-effects of ALG including the serum sickness of fever, rash and joint pains which may occur approximately 7 days after administration. Corticosteroids should not be used alone as they increase the risk of infection. Typically, if there is no response to ALG after 4 months a second course may be tried, prepared from another species. Overall, up to 80% of patients respond to combined ALG, steroids and ciclosporin.

2 *Ciclosporin* This is an effective agent which appears particularly valuable in combination with ALG and steroids.

3 *Androgens* These are beneficial in some patients with FA and acquired aplastic anaemia although an overall improved survival in acquired aplastic anaemia has not been proven. Oxymetholone is usually tried but side-effects are marked including virilization, salt retention and liver damage with cholestatic jaundice or rarely hepatocellular carcinoma. If there is no response in 4–6 months, androgens should be stopped. If there is a response the drug should be withdrawn gradually.

4 *Stem cell transplantation* Allogeneic transplantation offers the chance of permanent cure. For aplastic anaemia conditioning is with cyclophosphamide without irradiation but with ciclosporin which reduces the risks of graft failure and (with methotrexate) of graft-versus-host disease. The relative role of SCT versus immunosuppressive therapy in individual patients with aplastic anaemia is under constant review. In general terms, SCT is favoured in younger patients with severe aplastic anaemia and a human leucocyte antigen (HLA) matching sibling donor. Cure rates of up to 80% are obtained. However, non-myeloablative transplants (p. 262) are used in selected patients over the age of 40 years. SCT using unrelated volunteer donors and mismatched family members is achieving a survival ratio over 50% in patients with severe aplastic

anaemia. In older subjects and those with less severe disease, immunosuppression is usually tried first.
5 *Haemopoietic growth factors* Granulocyte colony-stimulating factor (G-CSF) may produce minor responses but does not lead to sustained improvement. Other growth factors have not proved helpful.

Red cell aplasia

Chronic form

This is a rare syndrome characterized by anaemia with normal leucocytes and platelets and grossly reduced or absent erythroblasts in the marrow (Fig. 20.3). The congenital form is known as Diamond–Blackfan syndrome (Table 20.3) and is inherited as a recessive condition. It is associated with a varying number of somatic disorders (e.g. of the face or heart). Mutation of a gene on chromosome 19 that encodes a ribosomal protein underlies some cases. Corticosteriods are the first line of treatment and SCT may be curative. Androgens may also produce improvement but side-effects on growth can be severe.

The acquired chronic form can occur without any obvious associated disease or precipitating factor (idiopathic), or may be seen with autoimmune diseases (especially systemic lupus erythematosus), with a thymoma, lymphoma or chronic lymphocytic leukaemia. Red cell aplasia from anti-erythropoietin antibodies has been rarely described in patients with chronic renal failure receiving recombinant erythropoietin. In some cases, immunosuppression with corticosteroids, ciclosporin, azathioprine or ALG is helpful. Monoclonal antibodies (e.g. Campath (anti-CD52) or rituximab (anti-CD20)) are being increasingly used in treatment of refractory acquired red cell aplasia and other autoimmune cytopenias.

If regular blood transfusions are needed, iron chelation therapy will also be necessary. SCT has been carried out in some severe cases.

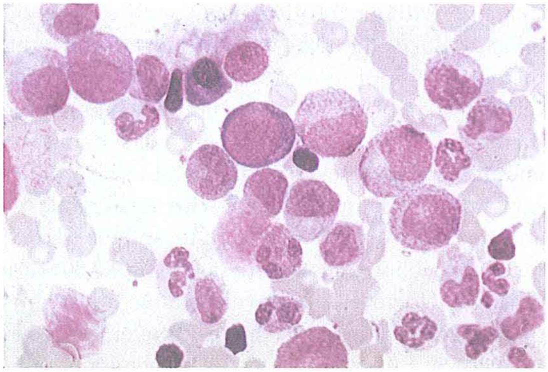


Fig. 20.3 The bone marrow in primary red cell aplasia. There is selective loss of erythropoiesis.

Transient form

Parvovirus B19 infects red cell precursors via the P antigen and causes a transient (5–10 days) red cell aplasia with the rapid onset of severe anaemia in patients with pre-existing shortened red cell survival (e.g. sickle cell disease or hereditary spherocytosis; Fig. 20.4). Transient red cell aplasia with anaemia may also occur in association with drug therapy (Table 20.3), and in normal infants or children, often with a history of a viral infection in the preceding 3 months.

Schwachman–Diamond syndrome (rare autosomal recessive syndrome)

This is a rare autosomal recessive syndrome characterized by varying degrees of cytopenia, especially neutropenia with a propensity to transform to myelodysplasia or acute myeloid leukaemia. Exocrine pancreatic dysfunction is an invariable feature while skeletal abnormalities, hepatic impairment and short stature are frequent.

Congenital dyserythropoietic anaemia

Congenital dyserythropoietic anaemias (CDAs) are a group of hereditary refractory anaemias characterized by ineffective erythropoiesis and erythroblast multinuclearity. The patient may be jaundiced with bone marrow expansion. The white cell and platelet counts are normal. The reticulocyte count is low for the degree of anaemia, despite increased marrow cellularity. The anaemia is of variable severity and is usually first noted in infancy or childhood. Iron overload may develop and splenomegaly is common. The CDAs have been classified into four types based on the degree to which megaloblastic changes, giant erythroblasts and dyserythropoietic changes

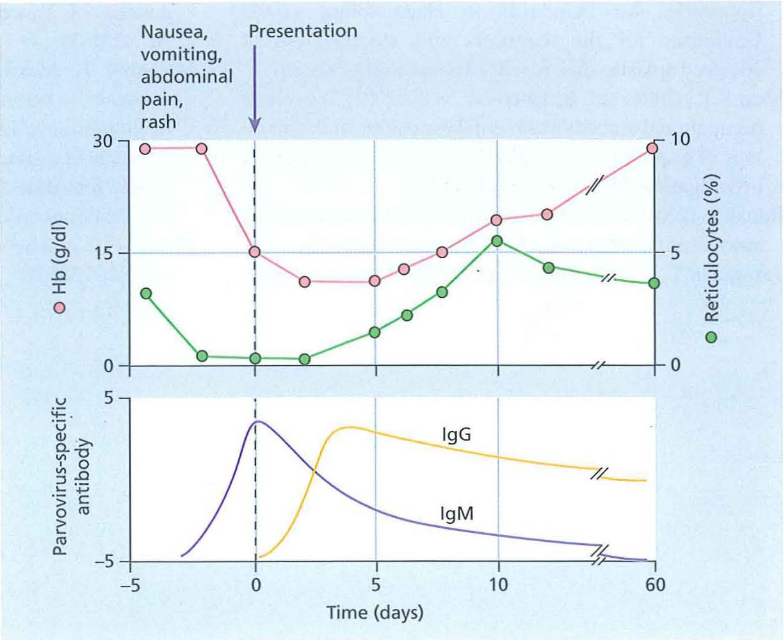


Fig. 20.4 Parvovirus infection: flow chart showing transient fall in haemoglobin and reticulocytes in a patient with hereditary spherocytosis.

Table 20.3 Classification of pure red cell aplasia.

Acute, transient	Chronic Congenital	Acquired
Parvovirus infection Infancy and childhood Drugs (e.g. azathioprine, co-trimoxazole)	Diamond–Blackfan syndrome	Idiopathic Associated with thymoma, systemic lupus erythematosus, rheumatoid arthritis, lymphoma, chronic lymphocytic leukaemia, T-large granular lymphocytosis, myelodysplasia, viral infection, drugs

are present. Type II is known as HEMPAS (hereditary erythroblast multinuclearity with a positive acidified serum lysis test) with some sera but not the patient's serum. The basic lesion in HEMPAS is a genetic defect in one of two enzymes, *N*-acetylglucosaminyltransferase or α-mannosidase, which are involved in glycosylation of several red cell membrane proteins. α-Interferon has induced remission in some cases.

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CHAPTER 21

21 Stem cell transplantation

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Principles of stem cell transplantation

Stem cell transplantation (SCT) involves eliminating a patient's haemopoietic and immune system by chemotherapy and/or radiotherapy and replacing it with stem cells either from another individual or with a previously harvested portion of the patient's own haemopoietic stem cells (Fig. 21.1). The term encompasses both ***bone marrow transplantation*** (BMT), in which stem cells are collected from bone marrow, and ***peripheral blood stem cell*** (PBSC) ***transplantation*** in which stem cells are collected from peripheral blood.

SCT may be *syngeneic* (from an identical twin), ***allogeneic*** (from another person) or ***autologous*** (from the patient's own stem cells) (Table 21.1).

The principal diseases for which SCT is performed are listed in Table 21.2. However, the exact role of SCT in the management of each disease is complex and depends on factors such as disease severity and subtype, remission status, age and, for allogeneic transplantation, availability of donors.

Collection of stem cells

Stem cells can be collected from bone marrow or from peripheral blood.

Bone marrow collection

The donor is given a general anaesthetic and 500–1200 mL of marrow is harvested from the pelvis. The marrow is heparinized and a mononuclear cell count is taken to assess the yield, which should be approximately $2–4 \times 10^8$ nucleated cells/kg body weight of the recipient.

Peripheral blood stem cell collection

PBSCs are taken using a cell-separator machine connected to the patient or donor via peripheral cannulae (Fig. 21.2). Blood is taken through one cannula and pumped around the machine where mononuclear cells are collected by centrifugation before the red cells are returned to the patient. This continuous process may take a few hours before enough mononuclear cells are collected.

Peripheral blood normally contains too few haemopoietic stem cells to allow collection of sufficient numbers for transplantation. Chemotherapy and growth factors can each increase the number by around 100 times. In future it is possible that stem cell populations may also be expanded *in vitro*.

Chemotherapy is used in patients undergoing autologous stem cell collection but not in healthy donors. PBSCs are usually collected during the recovery phase from a cycle of chemotherapy such as 1.5 g/m^2 of cyclophosphamide.

Granulocyte colony-stimulating factor (G-CSF) is given to patients or donors as a course of injections (typically 10 μg/kg/day for 4–6 days) until the white cell count starts to rise. PBSC collections are taken and, depending on the efficiency of stem cell

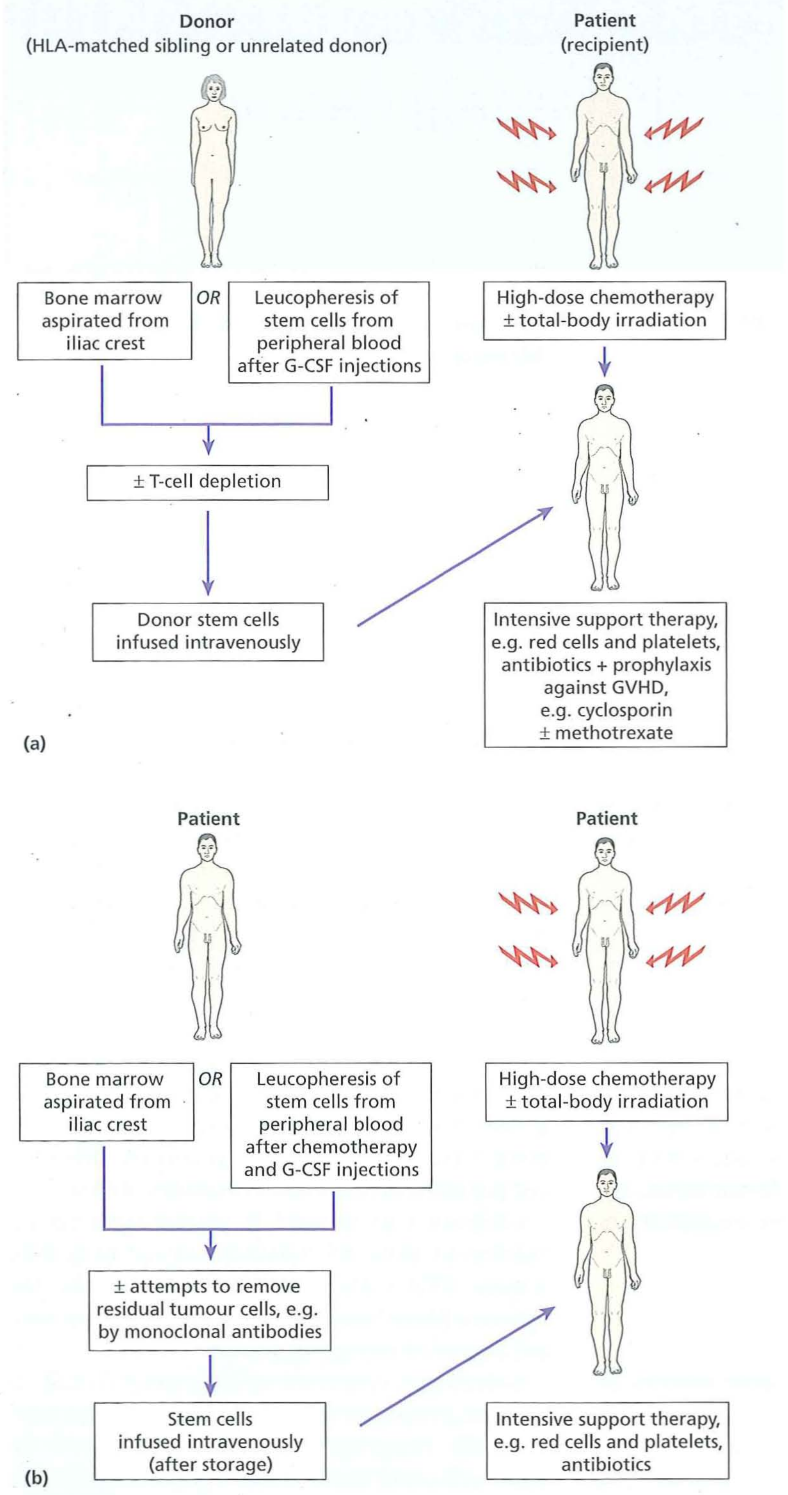


Fig. 21.1 Procedures for **(a)** allogeneic, and **(b)** autologous stem cell transplantation. G-CSF, granulocyte colony-stimulating factor; GVHD, graft-versus-host disease.

Table 21.1 Stem cell transplantation: potential donors.

HLA-matching sibling	Allogeneic
Unrelated HLA-matching volunteer	Allogeneic
Umbilical cord blood	Allogeneic
Identical twin	Syngeneic
Self	Autologous

HLA, human leucocyte antigen.

mobilization, repeated collections may be needed for up to 3 days. The adequacy of the collection may be assessed by:

1 $CD34^+$ cell count. Generally $>2.5 \times 10^6$/kg are needed for autologous transplantation.

2 *In vitro* colony assays, particularly granulocyte–macrophage colony-forming unit (CFU-GM) (p. 2), of which $1–5 \times 10^5$/kg would be considered adequate for transplantation.

Table 21.2 Stem cell transplantation: indications.

Allogeneic (or syngeneic)	Autologous
Acute lymphoblastic or myeloid leukaemia	Hodgkin's lymphoma and non-Hodgkin's lymphoma
Chronic myeloid leukaemia	Multiple myeloma
Other malignant disorders of the marrow (e.g. myelodysplasia, multiple myeloma, lymphoma, chronic lymphocytic leukaemia)	Acute and chronic leukaemias
Severe aplastic anaemia including Fanconi's anaemia	Severe autoimmune disease
Inherited disorders: thalassaemia major, sickle cell anaemia, immune deficiencies, inborn errors of metabolism in the haemopoietic and mesenchymal system (e.g. osteopetrosis)	Amyloidosis
Other acquired severe marrow diseases (e.g. paroxysmal nocturnal haemoglobinuria, red cell aplasia, myelofibrosis)	For 'gene therapy' of genetic disease (e.g. adenosine deaminase deficiency)

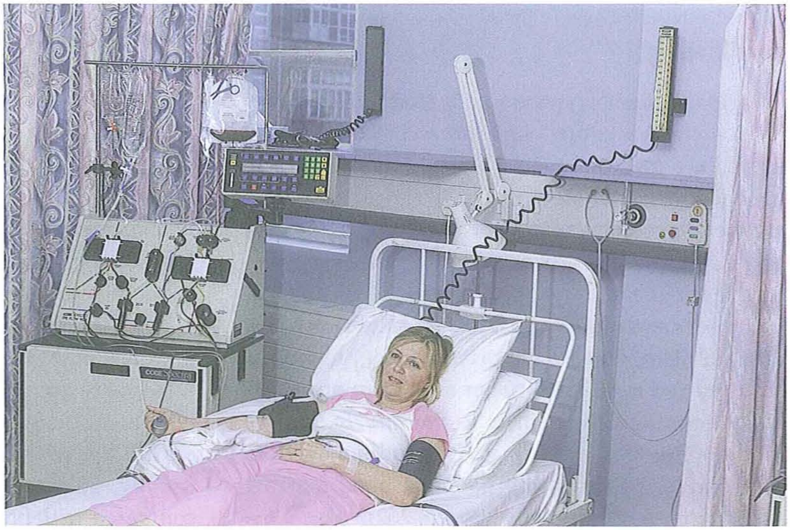


Fig. 21.2 Peripheral blood stem cell (PBSC) collection: a donor undergoing collection of PBSCs on a cell separator.

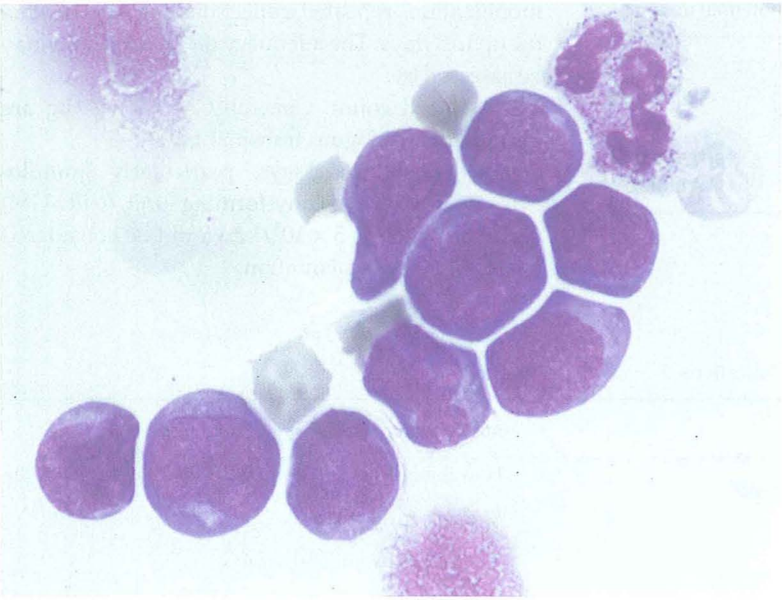


Fig. 21.3 Peripheral blood stem cell collection: enriched CD34$^+$ cells stained by May-Grünwald–Giemsa stain. The cells have the appearance of small and medium-sized lymphocytes.

Stem cell processing

After collection the stem cell harvest is processed with removal of red cells and concentration of the mononuclear cells. Autologous collections may be 'purged' by chemotherapy or antibodies in an attempt to remove residual malignant cells. Allogeneic collections may be treated with antibodies to remove T cells to reduce graft-versus-host disease (GVHD). CD34$^+$ stem cells may be selected from both types of harvest (Fig. 21.3).

Conditioning

Prior to infusion of haemopoietic stem cells patients receive chemotherapy, sometimes in combination with total body irradiation (TBI; Fig. 21.1) in a procedure called *conditioning*. This is designed to eradicate the patient's haemopoietic and immune system and, if present, malignancy. In addition, in the setting of allogeneic SCT it helps to suppress the host immune system and thereby prevent rejection of the 'foreign' stem cells. An important development that has occurred in SCT is a major shift from *myeloablative* conditioning regimes to *non-myeloablative* conditioning. Non-myeloabalative conditioning does not completely ablate the patient's bone marrow and is discussed more fully later (p. 262).

TBI is usually used in patients with malignant disease and is administered as a single dose or in smaller doses over several days (fractionated). The most commonly used drug is cyclophosphamide but busulfan, melphalan, cytosine arabinoside, etoposide or nitrosoureas are given in some protocols. At least 36 h are allowed for the elimination of the drugs from the circulation following the last dose of chemotherapy before donor stem cells are infused. Conditioning therapy is often complicated by mucositis and patients sometimes need parenteral nutrition. Trials are taking place in which monoclonal antibodies directed against specific antigens such as CD45 are attached to toxins or radioactive isotopes in an attempt to selectively target white cells as an aid to conditioning.

Post-transplant engraftment and immunity

After a period of typically 1–3 weeks of severe pancytopenia, the first signs of successful engraftment are monocytes and neutrophils in the blood with a subsequent increase in platelet count (Fig. 21.4). A reticulocytosis also begins and natural killer (NK) cells are among the earliest donor-derived lymphocytes to appear. G-CSF may be used to reduce the period of neutropenia. Engraftment is usually quicker following PBSC transplantation compared with BMT.

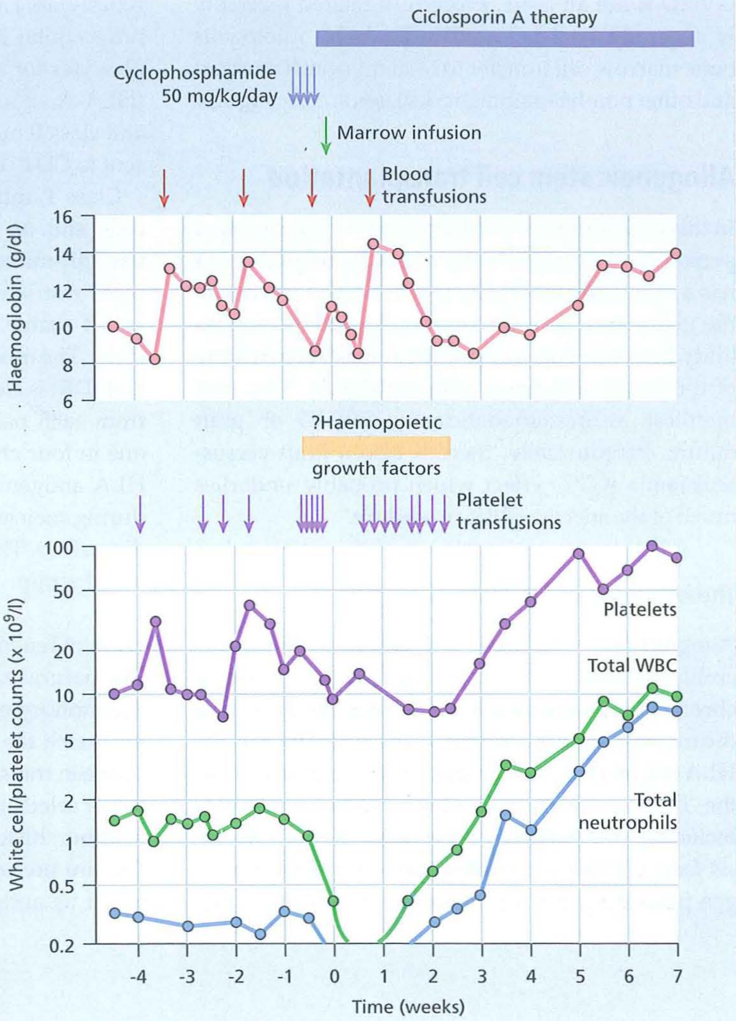


Fig. 21.4 Typical haematological chart of a patient undergoing allogeneic marrow transplantation for aplastic anaemia. WBC, white blood cells.

The marrow cellularity gradually returns to normal but the marrow reserve remains impaired for 1–2 years. There is profound immunodeficiency for 3–12 months with a low level of CD4 helper cells and a raised CD8 : CD4 ratio for 6 months or more. Immune recovery is quicker after autologous and syngeneic SCT than following allogeneic SCT. The patient's blood group changes to that of the donor and antigen-specific immunity becomes that of the donor after approximately 60 days.

Autologous stem cell transplantation

This allows the delivery of a high dose of chemotherapy, with or without radiotherapy, which otherwise would result in prolonged bone marrow aplasia. Stem cells are harvested and stored before the treatment is given and are then reinfused to 'rescue' the patient from the myeloablative effects of the treatment (Fig. 21.1). A limitation of the procedure is that tumour cells contaminating the stem cell harvest may be reintroduced into the patient. Nevertheless, autografting has a major role in the treatment of haematological diseases such as lymphoma and myeloma and is under investigation in other diseases such as acute leukaemia and severe autoimmune diseases. The major problem associated with autografting is recurrence of the original disease.

GVHD is not an issue. Procedure-related mortality is generally well below 5%. Trials of autologous bone marrow cell transfer to treat myocardial infarct and other non-haemopoietic diseases are in progress.

Allogeneic stem cell transplantation

In this procedure, stem cells harvested from another person are infused into the patient. The procedure has a significant morbidity and mortality and one of the major reasons is the immunological incompatibility between donor and patient despite matching of the human leucocyte antigens (HLA). This may manifest as immunodeficiency, GVHD or graft failure. Paradoxically, there is also a graft-versus-leukaemia (GVL) effect which probably underlies much of the success of the procedure.

The human leucocyte antigen system

Allografting would be impossible without the ability to perform HLA typing. The short arm of chromosome 6 contains a cluster of genes known as the major histocompatibility complex (MHC) or the HLA region (Fig. 21.5a). Genes in this region encode the HLA antigens and several other molecules including complement components, tumour necrosis factor (TNF) and proteins associated with antigen processing. HLA proteins are divided into two types: class I and II (Table 21.3). Their role is to bind intracellular peptides and 'present' these to T lymphocytes for antigen recognition. Class I molecules (HLA-A, -B and -C) present antigen to $CD8^+$ T cells and class II molecules (HLA-DR, -DQ and -DP) present to $CD4^+$ T cells (Fig. 21.5b).

Class I antigens are present on most nucleated cells and on the cell surface they are associated with β_2-microglobulin. Class II antigens have a more restricted tissue distribution and comprise α and β chains, both encoded by genes in the MHC locus. The inheritance of the four loci (HLA-A, -B, -C and -DR) is closely linked, one set of loci is inherited from each parent so that there is approximately a one in four chance of two siblings having identical HLA antigens (Fig. 21.6a). Crossing-over of genes during meiosis accounts for occasional unexpected disparities. The inheritance is independent of sex or blood group.

Human leucocyte antigen and transplantation

The natural role of HLA molecules is in directing T-lymphocyte responses and the greater the HLA mismatch the more severe is the immune response between transplanted cells. HLA typing is critical in donor selection for allogeneic SCT.

Minor histocompatibility antigens are peptides that are presented by HLA molecules and are able to act as antigens in SCT either because they are

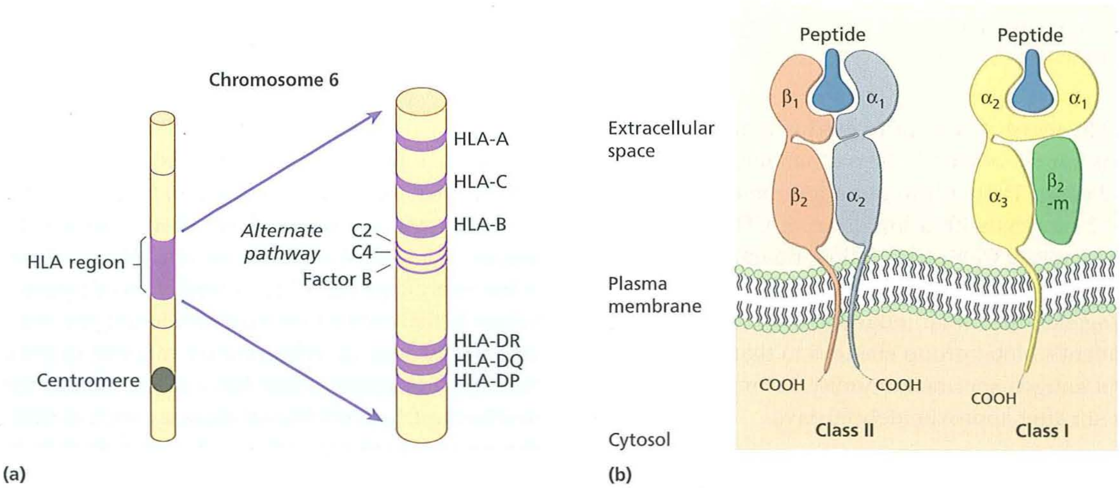


Fig. 21.5 **(a)** The human leucocyte antigen (HLA) complex. **(b)** HLA class I and II molecules showing protein domains and bound peptide.

Table 21.3 The human leucocyte antigens.

	Class I	Class II
Antigens	HLA-A, -B, -C	HLA-DR, -DP, -DQ
Distribution	All nucleated cells, platelets	B lymphocytes Monocytes Macrophages Activated T cells
Structure	Large polypeptide chain (MHC coded) and a β_2-microglobulin	Two polypeptide chains (α and β) both MHC coded
Interacts with	CD8 lymphocytes	CD4 lymphocytes

HLA, human leucocyte antigen; MHC, major histocompatibility complex.

polymorphic in the population or because they are encoded on the Y chromosome and therefore represent novel antigens when a female immune system engrafts in a male. They are likely to be important antigens in GVHD and the GVL reaction (see below).

HLA typing may be carried out by serological or molecular techniques. Serological testing involves the use of antibodies that are specific for individual HLA alleles or small families of alleles. Positivity may be detected by direct binding of a labelled antibody or by the use of complement to kill target cells that bind antibody (the two-stage lymphocytotoxicity test).

Molecular testing is performed on DNA. The most widely used methods use PCR sequence-specific primers or PCR sequence-specific oligonucleotide probes. Both methods use DNA primers or probes that react with polymorphic sequence motifs present within the nucleotide sequence of the *HLA* allele.

The nomenclature for *HLA* alleles is now standardized. A single antigenic specificity defined by serological typing (e.g. *HLA-A*) can be divided into different alleles by DNA sequencing. Each allele is given in numerical designation. The gene name is followed by an asterisk. The first two subsequent digits indicate the allele group. The third and fourth digits list subtypes. Subsequent digits indicate minor differences in non-coding regions. As an example, alleles at the *HLA-A* loci are written as *HLA-A*0101* to *HLA-A*8001*, where the first two digits after the asterisk indicate the type of the allele and the last two digits indicate the subtype. The type often corresponds to the serological antigen carried by the alleles (e.g. HLA-A2 for the *HLA-A*0201* to *HLA-A*0230* alleles). The nomenclature for the class II genes is similar but complicated by the fact that there may be more than one *HLA-DRB* gene on each chromosome (Fig. 21.6b).

Further cellular tests of histocompatibility that are sometimes employed, particularly in unrelated donor SCT, are limiting dilution analyses of cytotoxic T-lymphocyte or helper T-lymphocyte precursors (CTLp and HTLp). A higher value indicates greater mismatch and appears to correlate with increased GVHD.

When searching for an unrelated donor the aim is to match HLA-A, -B and -DR between recipient and donor and this is then called a 6/6 match. Occasionally a single mismatch (5/6) can be tolerated but it is rare to accept a donor with a greater mismatch than this. An exception is when a donor with only a single HLA haplotype match, usually a parent or sibling, is used in a ***haploidentical SCT***. Such transplants usually require a stem cell graft that is heavily depleted of T cells in order to limit the development of GVHD. There are over 4 million volunteer donors on international registries and the chance of identifying a matched unrelated donor for a patient lacking an HLA identical sibling (depending on the ethnic group) is usually greater than 50%.

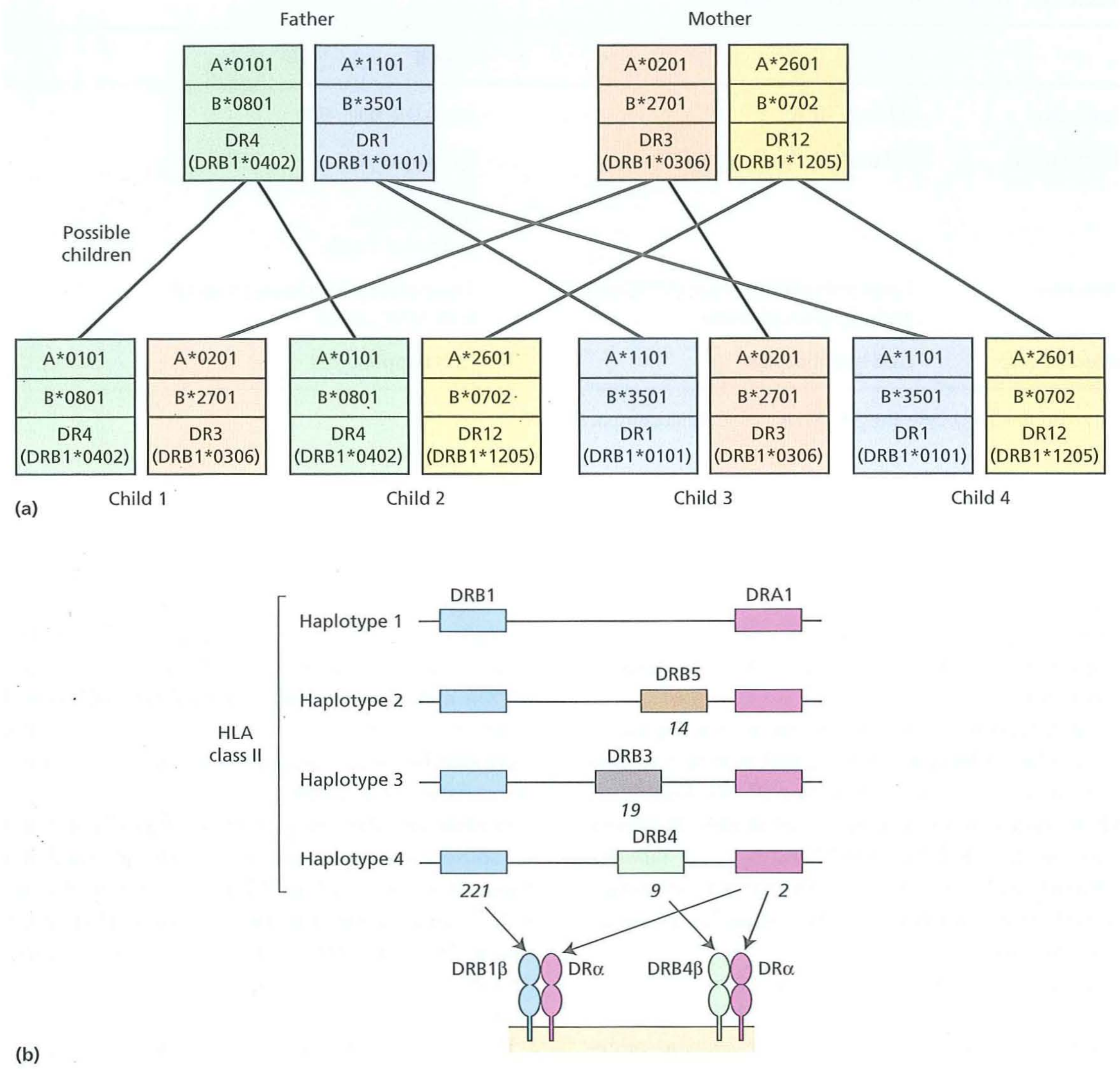


Fig. 21.6 **(a)** An example of the possible pattern of inheritance of the A, B and DR (*DRB1*) series alleles of the human leucocyte antigen (HLA) complex. **(b)** Molecular genetics of the HLA class II gene complex. There are four major haplotypes of MHC class II genes in the population and each individual may have up to two (one on each chromosome). The *DRA1* gene codes for the DRα protein and the *DRB1*, *DRB3*, *DRB4* and *DRB5* genes encode DRβ chains. Expression from the *DRB1* gene is higher than from the other genes. The number of alleles at each gene is shown underneath the gene in italics. Alleles at each locus have a standard nomenclature (e.g. the alleles at the *DRB1* gene are termed *DRB1*0101* to *DRB1*1608*). It is now known that the DR51, DR52 and DR53 antigens, which are defined by serological testing, are encoded from the *DRB5*, *DRB4* and *DRB3* genes, respectively.

Complications (Table 21.4)

Graft-versus-host disease

This is caused by donor-derived immune cells, particularly T lymphocytes, reacting against recipient tissues. Its incidence is increased with increasing age of donor and recipient and if there is any degree of HLA mismatch between them. GVHD prophylaxis is usually given as ciclosporin (intravenously or orally for 6–12 months) and methotrexate (three

Table 21.4 Complications of stem cell transplantation.

Early (usually <100 days)	Late (usually >100 days)
Infections, especially bacterial, fungal, herpes simplex virus, CMV	Infections, especially varicella-zoster, capsulate bacteria
Haemorrhage	Chronic GVHD (arthritis, malabsorption, hepatitis, scleroderma, sicca syndrome, lichen planus, pulmonary disease, serous effusions)
Acute GVHD (skin, liver, gut)	Chronic pulmonary disease
Graft failure	Autoimmune disorders
Haemorrhagic cystitis	Cataract
Interstitial pneumonitis	Infertility
Others: veno-occlusive disease, cardiac failure	Second malignancies

CMV, cytomegalovirus; GVHD, graft-versus-host disease.

Table 21.5 Acute graft-versus-host disease: clinical staging (Seattle system).

Stage	Skin	Liver (bilirubin, μmol/L)	Gut (diarrhoea, L/day)
I	Rash <25%	20–35	0.5–1.0
II	Rash 25–50%	35–80	1.0–1.5
III	Erythroderma	80–150	1.5–2.5
IV	Bullae, desquamation	>150	>2.5; severe pain, ileus

Fig. 21.7 Widespread erythematous skin rash in acute graft-versus-host disease following bone marrow transplantation.

or four injections). An alternative is to remove T cells from the donor stem cell infusion. In addition, anti-T-cell antibodies may be given to the patient.

In *acute GVHD*, occurring in the first 100 days, the skin, gastrointestinal tract or liver are affected (Table 21.5). The skin rash typically affects the face, palms, soles and ears but may in severe cases affect the whole body (Fig. 21.7). The diagnosis is usually confirmed by skin biopsy which shows initially single cell necrosis in the basal layer of the epidermis; lymphocyte infiltration may be scanty. Diarrhoea may lead to fluid and electrolyte depletion. Typically, bilirubin and alkaline phosphatase are raised but the other hepatic enzymes are relatively normal. Acute GVHD is usually treated by high doses of corticosteroids which are effective in the majority of cases.

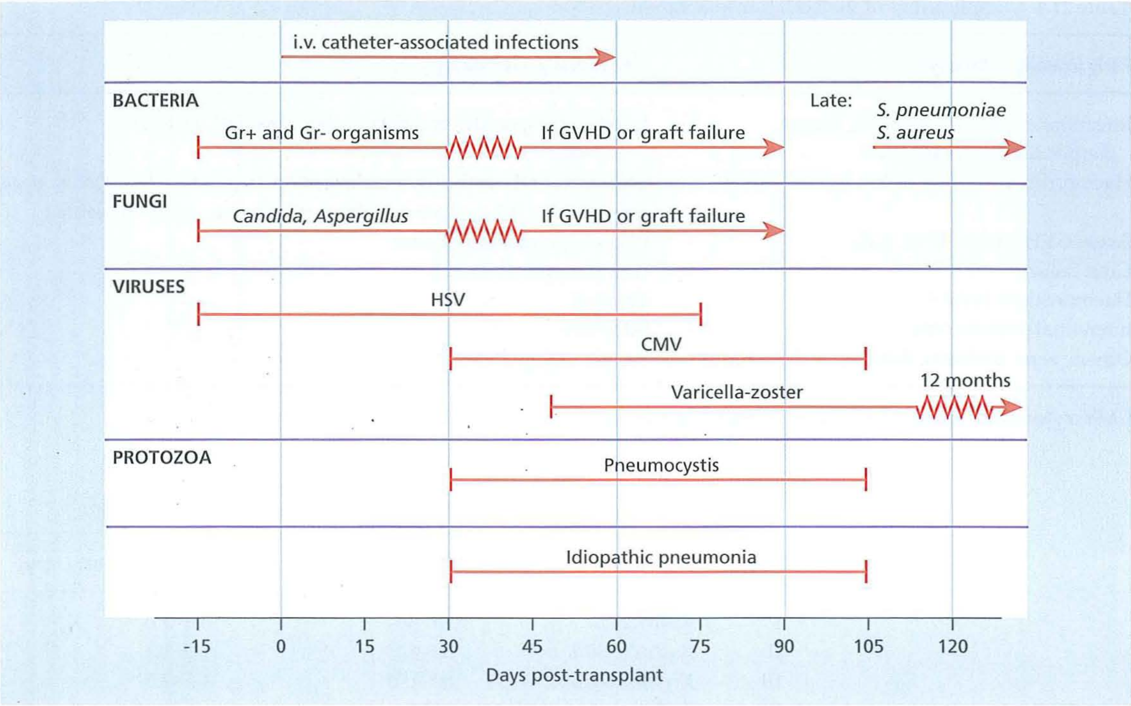


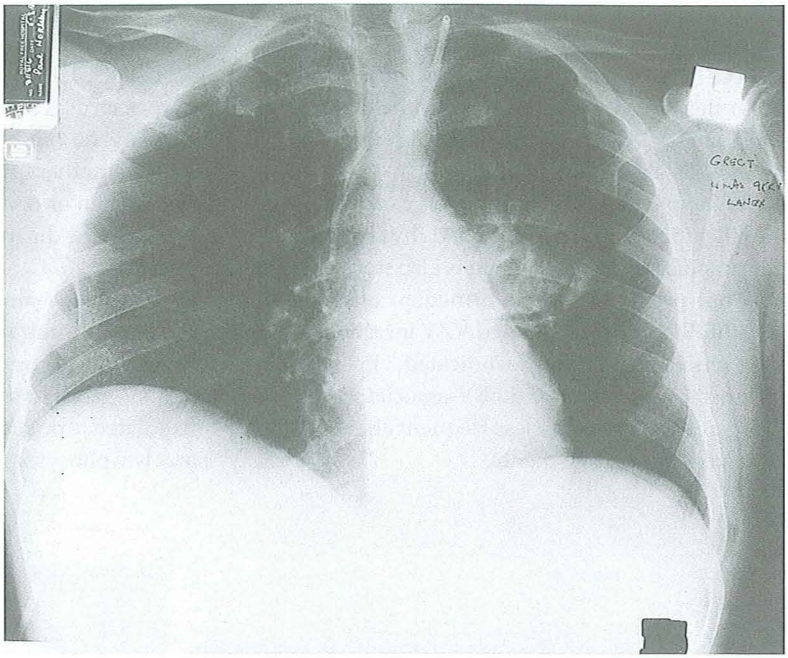
Fig. 21.8 Time sequence for development of different types of infection following allogeneic bone marrow transplantation. CMV, cytomegalovirus; Gr+, Gr–, Gram-positive or -negative; GVHD, graft-versus-host disease; HSV, herpes simplex virus.

In ***chronic GVHD***, occurring after 100 days and usually evolving from acute GVHD, these tissues are involved but also the joints and other serosal surfaces, the oral mucosa and lacrimal glands. Features of scleroderma, Sjögren's syndrome and lichen planus may develop. The immune system is impaired (including hyposplenism) with risk of infection. Malabsorption and pulmonary abnormalities are frequent. Drugs such as ciclosporin, azathioprine, mycophenolate mofetil, thalidomide or corticosteroids are used although response may be poor.

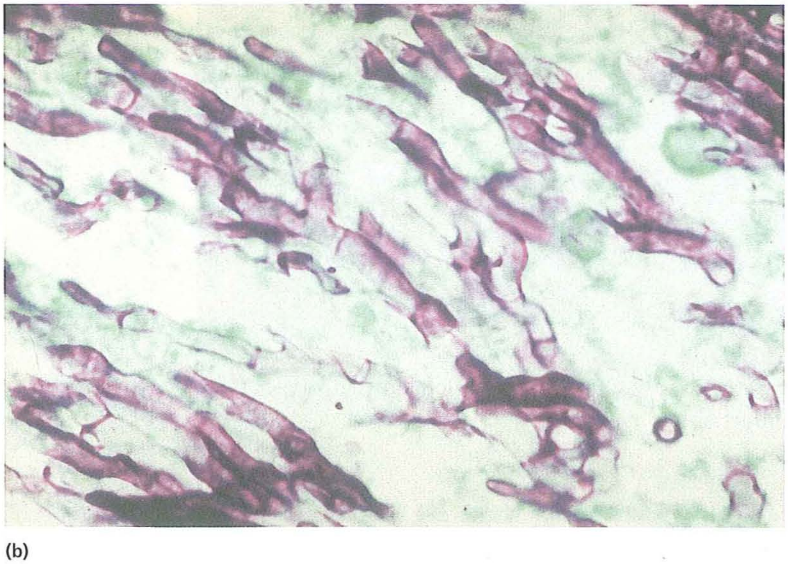
Infections

In the early post-transplant period, bacterial or fungal infections are frequent (Fig. 21.8). These may be reduced by reverse barrier nursing with laminar or positive pressure air flow and the use of skin and mouth antiseptics. Prophylactic therapy with aciclovir, antifungal agents and oral antibiotics is often added. If a fever or other evidence of an infection occurs, broad-spectrum intravenous antibiotics are commenced immediately after blood cultures and other appropriate microbiological specimens have been taken. Failure of response to antibacterial agents is usually an indication to commence systemic antifungal therapy with amphotericin B, caspofungin or voriconazole (p. 151). Fungal infections, especially *Candida* and *Aspergillus* species (Fig. 21.9) are a particular problem because of the prolonged neutropenia. Fluconazole is effective in reducing the risk of *Candida* infection and itraconazole may provide some prophylaxis against both organisms. The standard formulation of amphotericin is nephrotoxic and the newer preparations such as liposomal amphotericin are better tolerated.

Viral infections, particularly with the herpes group of viruses, are frequent with herpes simplex, cytomegalovirus (CMV) and varicella zoster virus (VZV) occurring at different peak intervals (Fig. 21.8).



(a)



(b)

Fig. 21.9 **(a)** Chest radiograph showing an aspergilloma in a patient following stem cell transplantation. **(b)** Cytology of sputum illustrates the branching septate hyphae of *Aspergillus* (methenamine silver stain).

CMV presents a particular threat and is associated with a potentially fatal interstitial pneumonitis as well as with hepatitis and falling blood counts. The infection may be caused by reactivation of CMV in the recipient or a new infection transmitted by the donor. In CMV-seronegative patients with CMV-seronegative donors, CMV-negative blood products or filtered blood must be given. Aciclovir may be useful in prophylaxis. Most centres screen patients regularly for evidence of CMV reactivation

following allogeneic transplantation using polymerase chain reaction (PCR) or antibody-based tests. If these tests become positive, ganciclovir may suppress the virus before disease occurs. Ganciclovir, foscarnet and CMV immunoglobulin may be tried for established CMV infection.

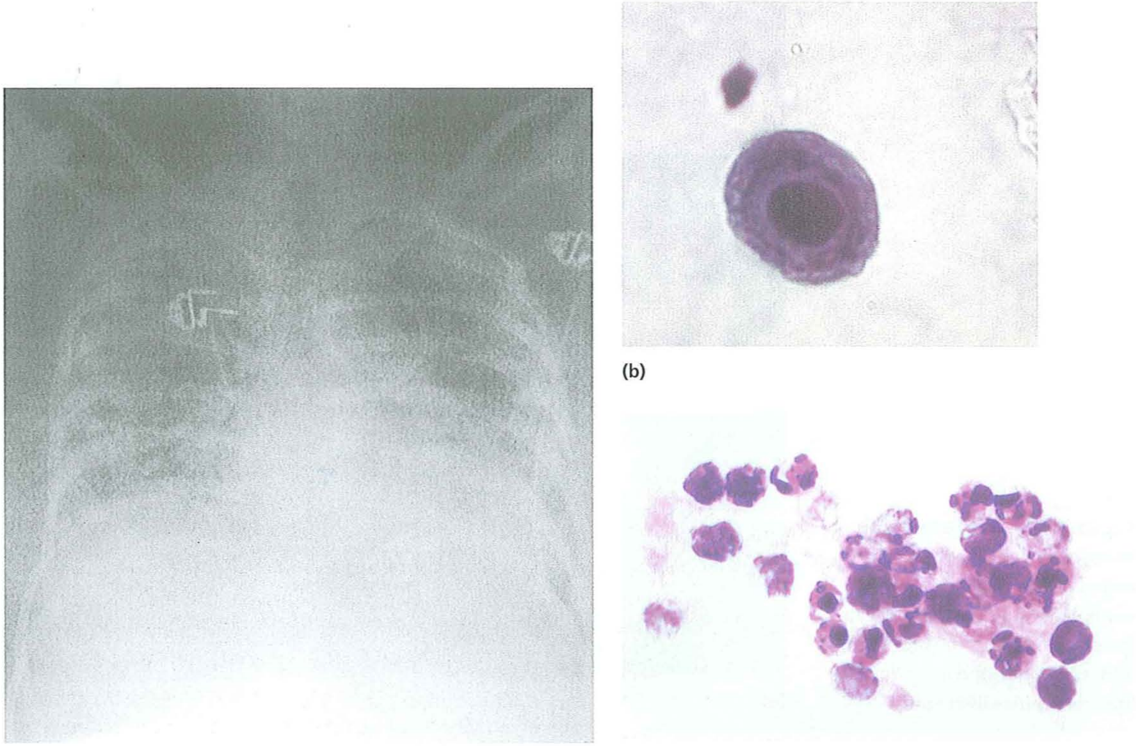
Pneumocystis carinii is another cause of pneumonitis that may be prevented by prophylactic co-trimoxazole. VZV infection is also frequent post-SCT but occurs later with a median onset at 4–5 months. Rarely, disseminated VZV infection occurs. Intravenous aciclovir is indicated. Epstein–Barr virus (EBV) infections and EBV-associated lymphoproliferative disease are less frequent after SCT than after solid organ transplants.

Interstitial pneumonitis

This is one of the most frequent causes of death post-SCT (Fig. 21.10). CMV is a frequent agent but other herpes viruses and *P. carinii* account for other cases; in many, no cause other than the previous radiation and chemotherapy can be implicated. Bronchoalveolar lavage or open lung biopsy may be needed to establish the diagnosis.

Blood product support

Platelet concentrates are given to maintain a count of 10×10^9/L or more. Platelets and blood transfusions given in the post-transplant period must be irradiated prior to administration in order to kill any lymphocytes that might cause GVHD.



(a) (b) (c)

Fig. 21.10 **(a)** Chest radiograph showing interstitial pneumonitis following bone marrow transplantation. Widespread diffuse mottling can be seen. The patient had received total body irradiation and had grade III graft-versus-host disease. No infective cause of the pneumonitis was identified. Possible causes include pneumocystis, cytomegalovirus, herpes zoster, fungal infection or a combination of these. **(b)** Sputum cytology: intranuclear CMV inclusion body in a pulmonary cell. Papanicolaou stain. **(c)** *Pneumocystis carinii* in bronchial washings, Gram–Weigert stain.

Other complications of allogeneic transplantation

Graft failure

The risk of graft failure is increased if the patient has aplastic anaemia or if T-cell depletion of donor marrow is used as GVHD prophylaxis. This suggests that donor T cells are needed to overcome host resistance to engraftment of stem cells.

Haemorrhagic cystitis

This is usually caused by the cyclophosphamide metabolite acrolein. Mesna is given in an attempt to prevent this. Certain viruses (e.g. adenovirus or polyomavirus) may also cause this complication.

Other complications

These include veno-occlusive disease of the liver (manifest as jaundice, hepatomegaly and ascites or weight gain) and cardiac failure as a result of the conditioning regime (especially high doses of cyclophosphamide) and previous chemotherapy on the heart. Haemolysis because of ABO incompatibility between donor and recipient may cause problems in the first weeks. Microangiopathic haemolytic anaemia may also occur.

Late complications

Relapse of the original disease (e.g. acute or chronic leukaemia) may occur. Bacterial infections are frequent, especially with Gram-negative or encapsulated organisms affecting the respiratory tract. Oral penicillin is given prophylactically to reduce this risk. VZV and fungal infections are also frequent. The use of prophylactic co-trimoxazole and oral aciclovir for 3–6 months reduces the risk of *Pneumocystis* and herpes infections, respectively.

Delayed pulmonary complications include restrictive pneumonitis and bronchiolitis obliterans. Endocrine complications include hypothyroidism, growth failure with low growth hormone levels in children, impaired sexual development and infertility. These endocrine problems are more marked if TBI has been used. Clinically apparent autoimmune disorders are infrequent and include myasthenia, rheumatoid arthritis, anaemia, thrombocytopenia or neutropenia. Autoantibodies are frequently detected in the absence of symptoms. Second malignancies (especially non-Hodgkin's lymphoma) occur with a six- or sevenfold incidence compared with controls. CNS complications include neuropathies and eye problems caused by chronic GVHD (sicca syndrome) or cataracts.

Umbilical cord transplants

Fetal blood contains a large number of haemopoietic stem cells and umbilical cord blood has been used successfully as a source of stem cells. The main problem is the small amount of blood and therefore limited number of stem cells that can be collected from each sample and this limits the application of umbilical blood transplants to children or small adults. *Ex vivo* expansion of progenitor cells or administration of two or more cord units may be of value in the future. The immunological properties of cord blood cells are under investigation.

Graft-versus-leukaemia effect and donor leucocyte infusions

After allogeneic transplantation the donor immune system helps to eradicate the patient's leukaemia, a phenomenon known as the ***graft-versus-leukaemia*** (GVL) effect. Evidence includes the decreased relapse rate in patients with severe GVHD, the increased relapse rate in identical twins and, most convincingly, the ability of ***donor leucocyte infusions*** (DLI) to cure relapsed leukaemia in some patients. Graft-versus-lymphoma and -myeloma effects also exist. The principle of DLI is that peripheral blood mononuclear cells are collected from the original allograft donor and directly infused into the patient at the time of leukaemia relapse (Fig. 13.6).

There is a large difference in the outcome of different diseases treated by DLI. Chronic myeloid leukaemia (CML) is most sensitive whereas acute lymphoblastic leukaemia rarely responds. In CML the response to DLI is better in cases of early relapse. PCR is used to monitor serial blood samples for evidence of recurrence of the *BCR-ABL* transcript before karyotypic or clinical relapse occurs (Fig. 13.6). DLI can then be used in cases of molecular relapse. The response to DLI may take several weeks but usually results in a permanent cure. The mechanism is unclear but a T-cell-mediated

Table 21.6 Vectors used in gene therapy.

Vector	Properties
Viral vectors	
Retroviral	Integrate into DNA; infect variety of cell types. Only infect dividing cells
Adenovirus	Do not integrate, therefore transient expression. Infect dividing and non-dividing cells. Often provoke immune response
Adeno-associated virus	Limited integration sites
Herpes virus	Can carry large genes; no integration. Can infect resting cells
Non-viral vectors	
Liposomal	Relatively simple and cheap to make
Naked DNA	Low efficiency of entry into cells
Ballistic (gene gun)	

alloreactive immune response is likely to be a major component.

Non-myeloablative transplants

In order to reduce the morbidity and mortality of allogeneic transplantation, a number of low-intensity ***non-myeloablative conditioning regimens*** have been introduced which do not completely destroy the host bone marrow. These can include agents such as fludarabine, low-dose irradiation, antilymphocyte globulin and low doses of busulfan or cyclophosphamide. The aim in these 'mini-transplants' is to use enough immunosuppression to allow donor stem cells to engraft without completely eradicating host marrow stem cells. DLIs are commonly used at a late stage in order to encourage complete donor engraftment. Such regimes rely heavily on the ability of the GVL effect to cure the underlying malignant disease (Fig. 21.11) and extend the age range and increase the treatment indications for allogeneic transplantation.

Gene therapy and SCT

The ability to introduce novel genes into cells using appropriate vectors offers the opportunity to manipulate stem cells before reinfusion. Gene therapy vectors may be viral or non-viral and their relative properties are shown in Table 21.6. Many different genes are being investigated for possible expression in cells such as haemopoietic stem cells and specific differentiated populations such as T lymphocytes. These include genes to correct specific inborn errors of metabolism, genes such as neomycin to 'mark' specific populations for subsequent analysis, 'suicide' genes such as thymidine kinase that render cells susceptible to ganciclovir and resistance genes to protect normal stem cells from high-dose chemotherapy. Unfortunately, haemopoietic stem cells are difficult to transfect because they are relatively rare and few are in cell cycle at any particular time. Therefore the sustained correction of metabolic disorders following the introduction of transduced autologous haemopoietic progenitors remains a difficult problem.

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CHAPTER 22

22 Platelets, blood coagulation and haemostasis

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The normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors (Fig. 22.1).

An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for

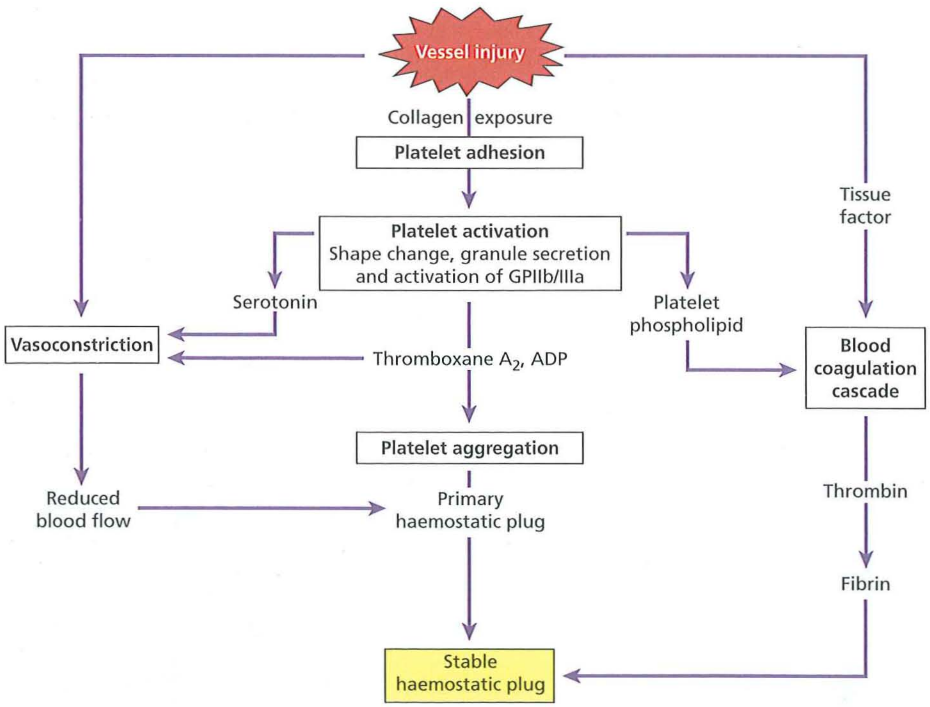

Fig. 22.1 The involvement of blood vessels, platelets and blood coagulation in haemostasis. ADP, adenosine diphosphate.

fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels. These are described later in the haemostatic response section on p. 275.

Components of haemostatic response

Platelets

Platelet production

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte—the megakaryoblast—arises by a process of differentiation from the haemopoietic stem cell (Fig. 22.2). The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. A picture of mature polyploid megakaryocytes is shown in Fig. 22.3. Very early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Mature megakaryocytes are extremely large, with an eccentric placed single lobulated nucleus and a low nuclear to cytoplasmic ratio. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000–5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days.

Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. Thrombopoietin increases the number and rate of maturation of megakaryocytes via c-Mpl receptor. Platelet levels start to rise 6 days after the start of therapy and remain high for 7–10 days. Unfortunately, thrombopoietin is not available for routine clinical practice. Platelets also have c-Mpl receptors for thrombopoietin and remove it from the circulation. Therefore, levels are high in thrombocytopenia as a result of marrow aplasia and vice versa.

The normal platelet count is approximately 250×10^9/L (range 150–400 $\times 10^9$/L) and the normal platelet lifespan is 7–10 days. Up to one-third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to 90% in cases of massive splenomegaly (p. 286).

Platelet structure

Platelets are extremely small and discoid, 3.0 × 0.5 μm in diameter, with a mean volume 7–11 fL. The ultrastructure of platelets is represented in

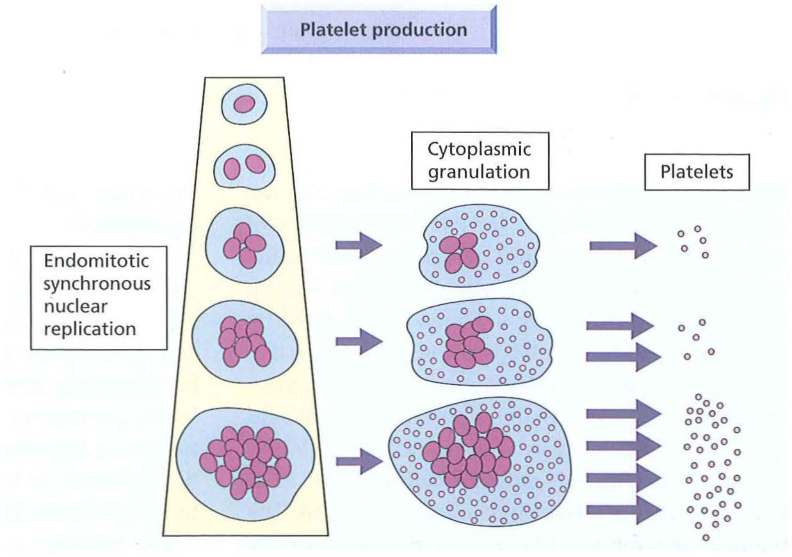

Fig. 22.2 Simplified diagram to illustrate platelet production from megakaryocytes.

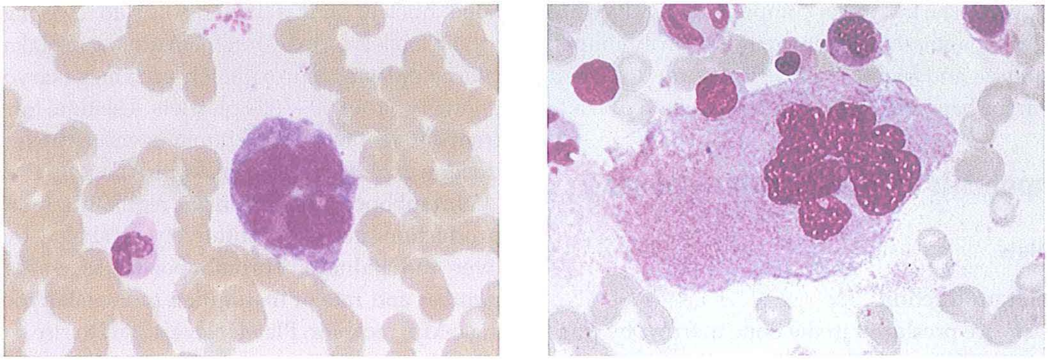

Fig. 22.3 Megakaryocytes: **(a)** immature form with basophilic cytoplasm; **(b)** mature form with many nuclear lobes and pronounced granulation of the cytoplasm.

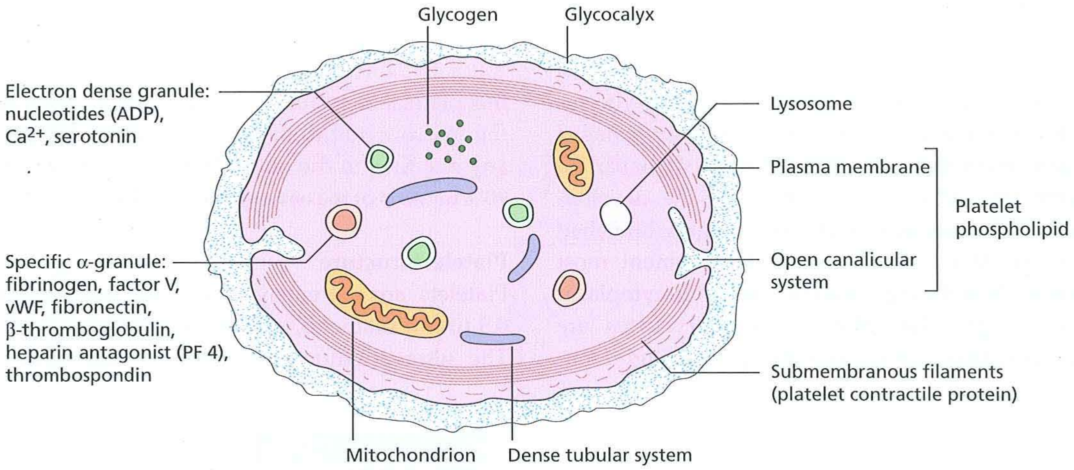

Fig. 22.4 The ultrastructure of platelets. ADP, adenosine diphosphate; PF, platelet factor; VWF, von Willebrand factor.

Fig. 22.4. The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein Ia (GPIa). Glycoproteins Ib (defective in Bernard–Soulier syndrome) and IIb/IIIa (defective in thrombasthenia) are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium (Fig. 22.5) where metabolic interactions occur (Fig. 22.6). The binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet–platelet aggregation.

The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa) (Fig. 22.7).

The platelet contains three types of storage

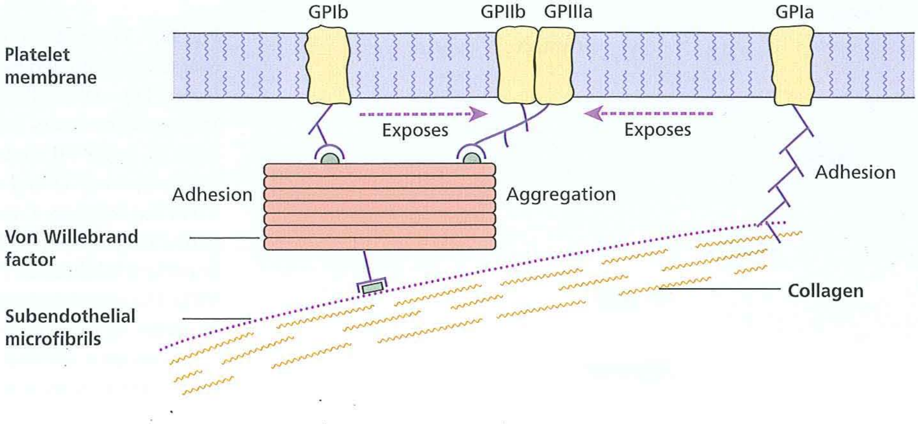

Fig. 22.5 Platelet adhesion. The binding of glycoprotein (GP) Ib (which consists of four proteins: GPIbα, GPIbβ, GPIX, GPV) to von Willebrand factor leads to adhesion to the subendothelium and also exposes the GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) binding sites to fibrinogen and von Willebrand factor leading to platelet aggregation. The GPIa site permits direct adhesion to collagen.

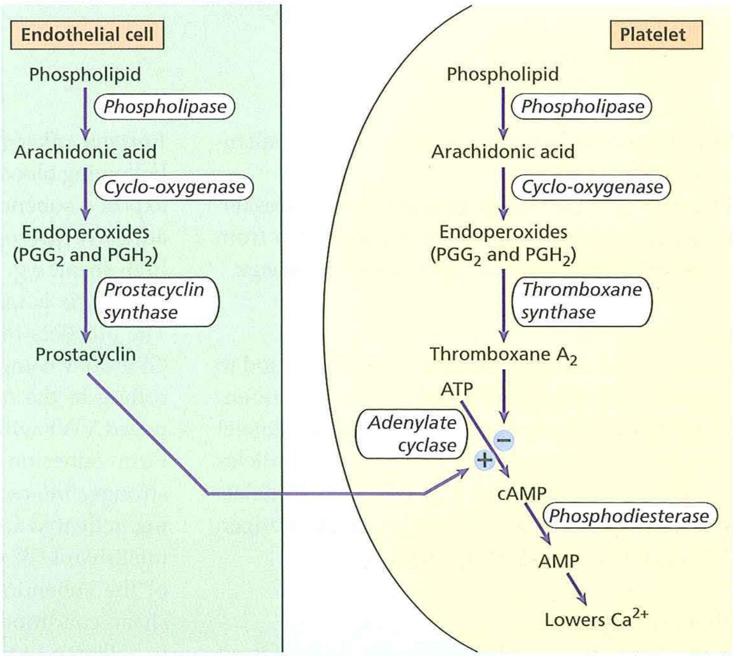

Fig. 22.6 The synthesis of prostacyclin and thromboxane. The opposing effects of these agents are mediated by changes in the concentration of cyclic adenosine monophosphate (cAMP) in platelets via stimulation or inhibition of the enzyme adenylate cyclase. cAMP controls the concentration of free calcium ions in the platelet which are important in the processes which cause adhesion and aggregation. High levels of cAMP lead to low free calcium ion concentrations and prevent aggregation and adhesion. ATP, adenosine triphosphate; Ca, calcium; PG, prostaglandin (G_2 and H_2).

granules: dense, α and lysosomes. The more frequent specific α granules contain a heparin antagonist (PF4), platelet-derived growth factor (PDGF), β-thromboglobulin, fibrinogen, VWF and other clotting factors. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. During the release reaction described below, the contents of

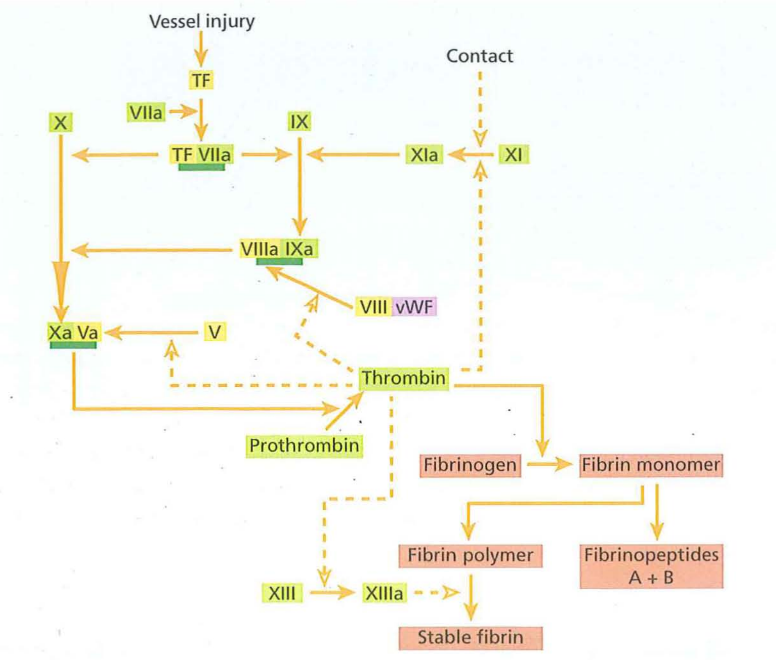

Fig. 22.7 The pathway of blood coagulation initiated by tissue factor (TF) on the cell surface. When plasma comes into contac with TF, factor VII binds to TF. The complex of TF and activate VII (VIIa) activates X and IX. TF pathway inhibitor (TFPI) is an important inhibitor of TF/VIIa. VIIIa–IXa complex greatly amplifies Xa production from X The generation of thrombin fro prothrombin by the action of Xa–Va complex leads to fibrin formation. Thrombin also activates XI (dashed line), V anc XIII. Thrombin cleaves VIII fron its carrier von Willebrand facto (VWF), greatly increasing the formation of VIIIa–IXa and hen of Xa–Va. Pale green, serine proteases; yellow, cofactors.

the granules are discharged into the open canalicular system.

Platelets are also rich in signalling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage.

Platelet antigens

Several platelet surface proteins have been found to be important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed a or b alleles (e.g. HPA-1a). Platelets also express ABO and human leucocyte antigen (HLA) class I but not class II antigens.

Platelet function

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet–vessel wall (adhesion) and platelet–platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions.

Platelet adhesion and activation

Following blood vessel injury, platelets adhere to t exposed subendothelial matrix proteins via speci adhesive glycoproteins (GP). Under condition high shear, e.g. arterioles, the exposed subendot lial matrix is initially coated with VWF multime The platelets than make contact with VWF via t GPIb-XI-V complex on platelets. This initiates plate rolling in the direction of blood flow over the posed VWF with activation of GPIIb/IIIa recepto Firm adhesion is established by the slower b stronger interaction of other glycoproteins inclu ing activated GPIIb/IIIa with VWF and GPVI a integrin $\alpha 1/\beta 2$ with collagen and other compone of the subendothelial matrix. Under static or lc shear conditions, platelets adhere predominan to collagen of the subendothelium. Collagen bin initially to GPIa/IIa, cross-links many of these in grin molecules, and in this way activates platelets

This ligand receptor binding results in a compl cascade of signals which result in platelet activati The events that follow are shape change and sprea ing, activation of GPIIb/IIIa and granule secreti Platelets become more spherical and extrude lo pseudopodia which enhance platelet vessel w

and platelet–platelet interaction. The end result of spreading is a flattened spread out platelet with granules and organelles in the centre, resulting in a characteristic fried egg appearance. These changes are brought about by the actin cytoskeleton. The granules are secreted from the centre of the cell.

Von Willebrand factor VWF is involved in platelet adhesion to the vessel wall and to other platelets (aggregation) (Fig. 22.5). It also carries factor VIII (see below) and used to be referred to as factor VIII-related antigen (VIII-Rag). It is a large cysteine-rich glycoprotein, with multimers made up on average of 2–50 subunits, with a molecular weight (MW) of $0.8–20 \times 10^6$. VWF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weiberl–Palade bodies and platelet α granules respectively. Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel–Palade bodies. The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of desmopressin (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from Weibel–Palade bodies is in the form of large and ultra large multimers, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multimers by the specific plasma metalloprotease, ADAMTS-13.

Platelet aggregation

It is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has about 50–80 000 GPIIb/IIIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, due to binding of α-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb/IIIa, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm connection and further activation of the platelet.

Platelet release reaction and amplification

Primary activation by various agonists induces intracellular signalling, leading to the release of α and δ- granules. α-Granule contents play an important role in platelet aggregate formation and stabilization and, in addition, the ADP released from dense granules plays a major positive feedback role in promoting platelet activation. TXA2 is the second of the two major platelet positive feedback loops important in secondary amplification of platelet activation to firm a stable platelet aggregate. It is formed *de novo* upon activation of cytosolic phospholipase A_2 (PL_{A2}) which is the rate limiting step. This liberates arachidonic acid from the membrane phospholipids, and is metabolized by cycloxogenase to TXA2. It is a labile substance and lowers platelet cyclic adenosine monophosphate (cAMP) levels and initiates the release reaction (Fig. 22.6). Thromboxane A_2 not only potentiates platelet aggregation, but also has powerful vasoconstrictive activity. The release reaction is inhibited by substances that increase the level of platelet cAMP. One such substance is the prostaglandin prostacyclin (PGI_2) which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium.

Clot formation and retraction

The highly localized enhancement of ongoing platelet activation by ADP and TXA2 results in a platelet plug large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely degranulated and adherent to each other. This is followed by clot retraction which is mediated by GPIIb/IIIa receptors which link the cytoplasmic actin filaments to the surface bound fibrin polymers.

Platelet procoagulant activity

After platelet aggregation and release, the exposed membrane phospholipid (platelet factor 3) is available for two reactions in the coagulation cascade. Both phospholipid-mediated reactions are calcium-ion dependent. The first (tenase) involves factors IXa, VIIIa and X in the formation of factor Xa (Fig. 22.7). The second (prothrombinase) results in the formation of thrombin from the interaction of factors Xa, Va and prothrombin (II). The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins.

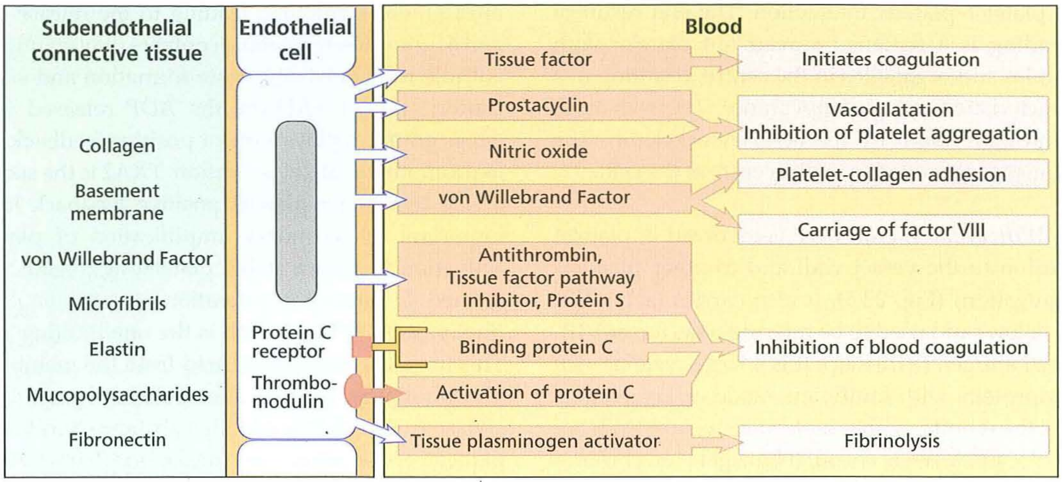

Fig. 22.8 The endothelial cell forms a barrier between platelets and plasma clotting factors and the subendothelial connected tissues. Endothelial cells produce substances that can initiate coagulation, cause vasodilatation, inhibit platelet aggregation or haemostasis, or activate fibrinolysis.

Growth factor

PDGF found in the specific granules of platelets stimulates vascular smooth muscle cells to multiply and this may hasten vascular healing following injury.

Natural inhibitors of platelet function

Nitric oxide (NO) is constitutively released from endothelial cells and also from macrophages and platelets. It has a short half-life of 3–5 s. It inhibits platelet activation and promotes vasodilatation. Prostacyclin synthesized by endothelial cells also inhibits platelet function (Fig. 22.8) and causes vasodilatation by raising cyclic guanosine monophosphate (GMP) levels. The transmembrane protein PECAM-1 is expressed also on endothelial cells. It is its own ligand and inhibits platelet activation by collagen.

Blood coagulation

The coagulation cascade

Blood coagulation involves a biological amplification system in which relatively few initiation substances sequentially activate by proteolysis a cascade of circulating precursor proteins (the coagulation factor enzymes) which culminates in the generation of thrombin; this, in turn, converts soluble plasma fibrinogen into fibrin (Fig. 22.7). Fibrin enmeshes the platelet aggregates at the sites of vascular injury and converts the unstable primary platelet plugs to firm, definitive and stable haemostatic plugs. A list of the coagulation factors appears in Table 22.1. They are assembled from a small number of molecules or domains (Fig. 22.9). The operation of this enzyme cascade requires local concentration of circulating coagulation factors at the site of injury.

Surface-mediated reactions occur on exposed collagen, platelet phospholipid and tissue factor. With the exception of fibrinogen, which is the fibrin clot subunit, the coagulation factors are either enzyme precursors or cofactors (Table 22.1). All the enzymes, except factor XIII, are serine proteases (i.e. their ability to hydrolyse peptide bonds depends upon the amino acid serine at their active centre; Fig. 22.10). The scale of amplification achieved in this system is dramatic, (e.g. 1 mol of activated factor XI through sequential activation of factors IX, X and prothrombin may generate up to 2×10^8 mol of fibrin).

Coagulation *in vivo*

The generation of thrombin *in vivo* is a complex network of amplification and negative feedback loops

Table 22.1 The coagulation factors.

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor*
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutaminase
	Prekallikrein (Fletcher factor)	Serine protease
	HMWK (Fitzgerald factor)	Cofactor*

HMWK, high molecular weight kininogen.
* Active without proteolytic modification.

to ensure a localized and limited production. The generation of thombin is dependant on three enzyme complexes, each consisting of protease, cofactor, phospholipids (PL) and calcium. They are extrinsic Xase (VIIa, TF, PL, Ca^{2+}) and intrinsic Xase (IXa, VIIIa, PL, Ca^{2+}) generating FXa, prothrombinase complex (Xa, Va, PL, Ca^{2+}) generating thrombin. The generation of thrombin following vascular injury occurs in two waves of very different magnitude with different functions. During the initiation phase small amounts are generated (picomolar concentrations) which prepares the coagulation cascade for the second larger thrombin burst in the amplification when micromolar concentrations are produced, i.e. a million-fold higher concentration than produced during the initiation phase.

Initiation

Coagulation is initiated by the interaction of the membrane bound TF exposed by vascular injury, with plasma factor VIIa. One to two per cent of the total factor VII circulates in the activated form, but does not express proteolytic activity unless bound to TF. The factor VIIa-tissue factor (extrinsic factor Xase) complex activates both factor IX and factor X. The factor Xa, in the absence of its cofactor, forms small amounts of thrombin from prothrombin. This is insufficient to initiate significant fibrin polymerization, it activates the coenzymes, factor V and factor VIII, platelets and factor XI.

Amplification

The initiation pathway or extrinsic Xase is rapidly inactivated by TFPI which forms a quaternary complex of VIIa, TF, Xa and TFPI. The thrombin generation is now dependant on the traditional intrinsic pathway which has been primed by the small amounts of thrombin generated during initiation. In the amplification phase the intrinsic Xase formed by IXa and VIIIa on phospholipid surface in the presence of Ca^{2+} activates sufficient Xa which then in combination with Va, PL and Ca^{2+} forms the prothrombinase complex and results in the explosive generation of thrombin which acts on fibrinogen to form the fibrin clot.

In the 'classic' pathway formulated to explain *in vitro* coagulation testing, initiation of the pathway required contact reactions between factor XII, kallikrein and high molecular weight kininogen (HMWK) leading to the activation of factor XI. However, the lack of abnormal bleeding in individuals with hereditary deficiencies of these contact factors suggests that these reactions are not required for physiological coagulation *in vivo*.

Factor XI does not seem to have a role in the physiological initiation of coagulation. It has a

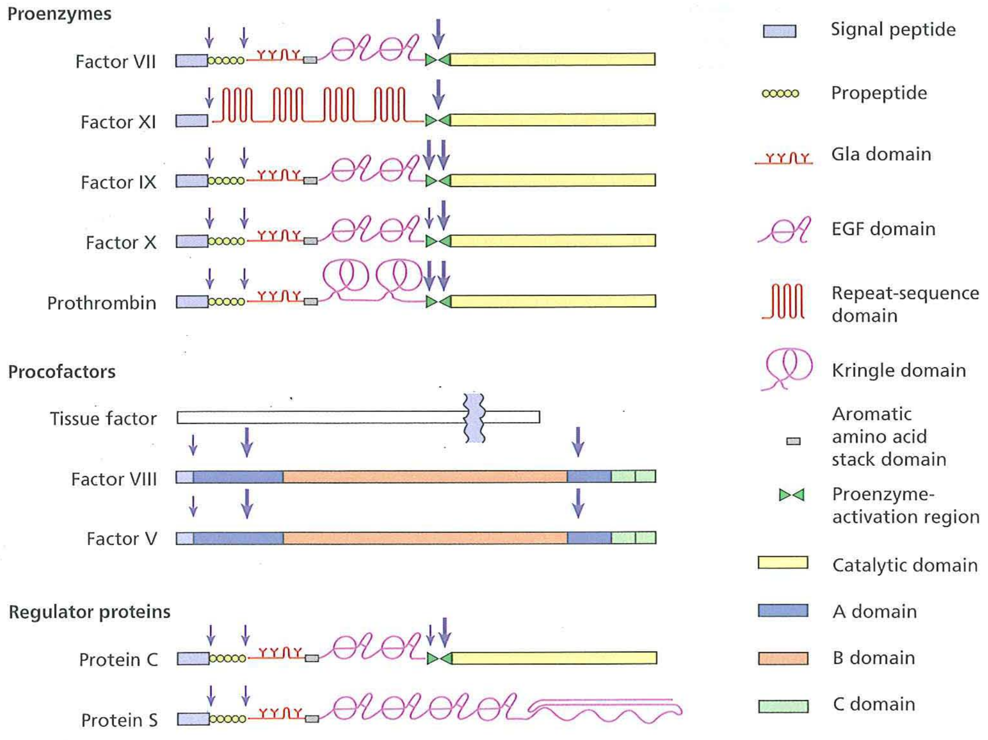

Fig. 22.9 Domains of the enzymes, receptors and cofactors involved in blood coagulation and regulation. The components of blood coagulation are proenzymes, procofactors and regulator proteins. The proenzymes, including protein C, contain a catalytic domain, an activation region and a signal peptide. The vitamin K-dependent proteins include a propeptide and a γ-carboxyglutamic acid (Gla) domain. Other important domains include the epidermal growth factor-like (EGF) domain, the kringle domain and the repeat-sequence domain. Tissue factor is an integral membrane protein unrelated to other known proteins. Factors V and VIII have marked similarities in structure. Sites of intracellular peptide bonds cleaved during synthesis are indicated by thin arrows, and sites of peptide bonds cleaved during protein activation are indicated by thick arrows. The transmembrane domain of tissue factor is shown within the phospholipid bilayer. (From B. Furie and B.C. Furie 1992, courtesy of the *New England Journal of Medicine* with permission.)

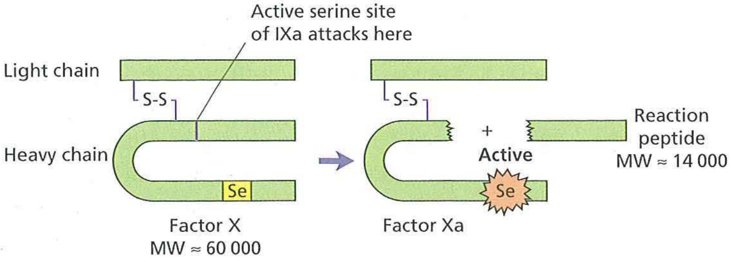

Fig. 22.10 Serine (Se) protease activity. This example shows the activation of factor X by factor IX.

supplementary role in the activation of factor IX (see above) and may be important at major sites of trauma or at operations and potentially causes excess bleeding in factor XI deficient individuals.

Thrombin hydrolyses fibrinogen, releasing fibrinopeptides A and B to form fibrin monomers (Fig. 22.11). Fibrin monomers link spontaneously by hydrogen bonds to form a loose insoluble fibrin

Table 22.2 The coagulation factors.

Factor	Plasma half-life (h)	Plasma concentration (mg/L)	Comments
II	65	100	Prothrombin group: vitamin K needed for synthesis; require Ca^{2+} for activation
VII	5	0.5	
IX	25	5	
X	40	10	
I	90	3000	Thrombin interacts with them; increase in inflammation, pregnancy, oral contraceptives
V	15	10	
VIII	10	0.1	
XI	45	5	
XII	50	30	

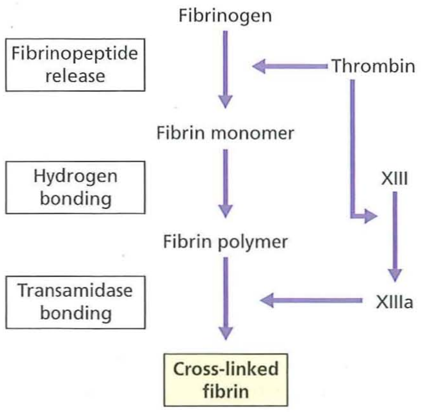

Fig. 22.11 The formation and stabilization of fibrin.

polymer. Factor XIII is also activated by thrombin together with calcium. Activated factor XIII stabilizes the fibrin polymers with the formation of covalent bond cross-links.

Fibrinogen has a MW 340 000 and consists of two identical subunits, each containing three dissimilar polypeptide chains (Aα, Bβ and γ) which are linked by disulphide bonds. After cleavage by thrombin of fibrinopeptides A and B, fibrin monomer consists of three paired α, β and γ chains.

Some of the properties of the coagulation factors are listed in Table 22.2. The activity of factors II, VII, IX and X is dependent upon vitamin K which is responsible for carboxylation of a number of terminal glutamic acid residues on each of these molecules (see Fig. 24.7).

The serine protease coagulation factors along with those of the fibrinolytic system (see below) have a high degree of homology and contain characteristic structural domains (Fig. 22.9), such as the kringles which are concerned with substrate binding and the carboxylated glutamic acid (Gla) residues which bind to phospholipid. There are also regions of homology with fibronectin (finger regions) and with epidermal growth factor. Although factor VIII and V cofactors are not protease enzymes, they circulate in a precursor form that requires limited cleavage by thrombin for expression of full cofactor activity.

Physiological limitation of blood coagulation

Unchecked blood coagulation would lead to dangerous occlusion of blood vessels (thrombosis) if the protective mechanisms of coagulation factor inhibitors, blood flow and fibrinolysis were not in operation.

Coagulation factor inhibitors

It is important that the effect of thrombin is limited to the site of injury. The first inhibitor to act is tissue factor pathway inhibitor (TFPI) which is synthesized in endothelial cells and is present in plasma and platelets and accumulates at the site of injury caused by local platelet activation. This inhibits Xa and VIIa and tissue factor to limit the main *in vivo* pathway by forming the quaternary complex. There is direct inactivation of thrombin and other serine protease factors by other circulating inhibitors of which antithrombin is the most potent. It inactivates serine proteases by combining with them by peptide bonding to form high molecular weight stable complexes

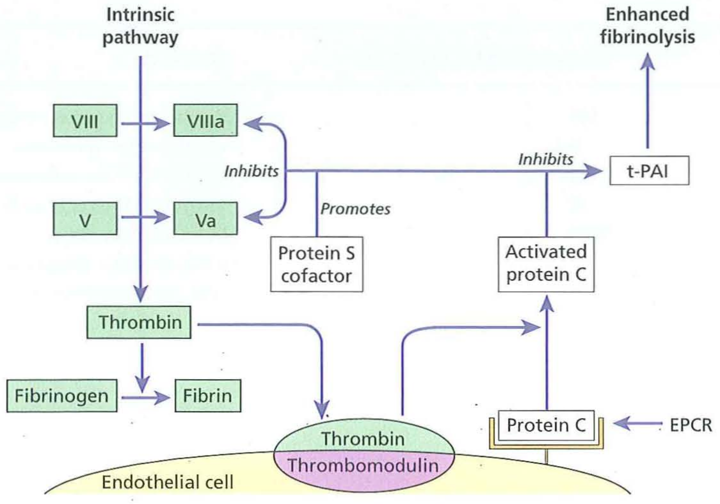

Fig. 22.12 Activation and action of protein C by thrombin which has bound to thrombomodulin on the endothelial cell surface. Protein S is a cofactor that facilitates binding of activated protein C to the platelet surface. The inactivation of factors Va and VIIIa results in the inhibition of blood coagulation. The inactivation of tissue plasminogen activator inhibitor (tPAI) enhances fibrinolysis. EPCR, endothelial protein C receptor.

(Fig. 25.6). Heparin potentiates its action markedly. Another protein, heparin cofactor II, also inhibits thrombin. α_2-Macroglobulins, α_2-antiplasmin, C_1 esterase inhibitor and α_1-antitrypsin also exert inhibitory effects on circulating serine proteases.

Protein C and protein S

These are inhibitors of coagulation cofactors V and VIII. Thrombin binds to an endothelial cell surface receptor, thrombomodulin. The resulting complex activates the vitamin K-dependent serine protease protein C which is able to destroy activated factors V and VIII, thus preventing further thrombin generation. The action of protein C is enhanced by another vitamin K-dependent protein, S, which binds protein C to the platelet surface (Fig. 22.12). An endothelial protein C receptor localizes protein C to the endothelial surface, promoting protein C activation by the thrombin–thrombomodulin complex (Fig. 22.12). In addition, activated protein C enhances fibrinolysis (Fig. 22.12).

As with other serine proteases, activated protein C is subject to inactivation by serum protease inactivators (serpins), e.g. antithrombin.

Blood flow

At the periphery of a damaged area of tissue, blood flow rapidly achieves a dilution and dispersal of activated factors before fibrin formation has occurred. Activated factors are destroyed by liver parenchymal cells and particulate matter is removed by liver Kupffer cells and other reticuloendothelial cells.

Fibrinolysis

Fibrinolysis (like coagulation) is a normal haemostatic response to vascular injury. Plasminogen, a β-globulin proenzyme in blood and tissue fluid, is converted to the serine protease plasmin by activators either from the vessel wall (intrinsic activation) or from the tissues (extrinsic activation) (Fig. 22.13). The most important route follows the release of tissue plasminogen activator (tPA) from endothelial cells. tPA is a serine protease that binds to fibrin. This enhances its capacity to convert thrombus-bound plasminogen into plasmin. This fibrin dependence of tPA action strongly localizes plasmin generation by tPA to the fibrin clot. Release of tPA

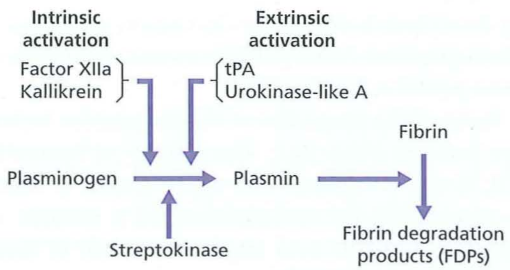

Fig. 22.13 The fibrinolytic system. tPA, tissue plasminogen activator.

occurs after such stimuli as trauma, exercise or emotional stress. Activated protein C stimulates fibrinolysis by destroying plasma inhibitors of tPA (Fig. 22.12). On the other hand, thrombin inhibits fibrinolysis by activating thrombin-activated fibrinolysis inhibitor (TAFI).

Plasmin generation at the site of injury limits the extent of the evolving thrombus. The split products of fibrinolysis are also competitive inhibitors of thrombin and fibrin polymerization. Normally, α_2-antiplasmin inhibits any local free plasmin.

Fibrinolytic agents are widely used in clinical practice (p. 317). Therapeutic recombinant tPA has been synthesized using recombinant DNA technology. The bacterial agent streptokinase is a peptide produced by haemolytic streptococci and forms a complex with plasminogen, which converts other plasminogen molecules to plasmin. Urokinase is a tPA initially isolated from human urine.

Plasmin is capable of digesting fibrinogen, fibrin, factors V and VIII and many other proteins. Cleavage of peptide bonds in fibrin and fibrinogen produces a variety of split (degradation) products (Fig. 22.13). Large amounts of the smallest fragments D and E can be detected in the plasma of patients with disseminated intravascular coagulation (p. 298).

Inactivation of plasmin

Tissue plasminogen activator is inactivated by plasminogen activator inhibitor (PAI). Circulating plasmin is inactivated by potent inhibitors α_2-antiplasmin and α_2-macroglobulin.

Endothelial cells

The endothelial cell has an active role in the maintenance of vascular integrity. This cell provides the basement membrane that normally separates collagen, elastin and fibronectin of the subendothelial connective tissue from the circulating blood (Fig. 22.8). Loss or damage to the endothelium results in both haemorrhage and activation of the haemostatic mechanism. The endothelial cell also has a potent inhibitory influence on the haemostatic response, largely through the synthesis of PGI_2 and NO, which have vasodilatatory properties and inhibit platelet aggregation. In contrast, endothelins are a family of vasoactive peptides that can activate fibrinolysis via the release of tPA. Synthesis of tissue factor which initiates haemostasis only occurs in endothelial cells following activation and its natural inhibitor, tFPI, is also synthesized. Synthesis of prostacyclin, VWF, plasminogen activator, antithrombin and thrombomodulin, the surface protein responsible for activation of protein C, provides agents that are vital to both platelet reactions and blood coagulation (Fig. 22.8).

Haemostatic response

Vasoconstriction

An immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. When there is widespread damage this vascular reaction prevents exsanguination. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thromboxane A_2 liberated from platelets, and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity (Fig. 22.1).

Platelet reactions and primary haemostatic plug formation

Following a break in the endothelial lining, there is an initial adherence of platelets to exposed connective tissue, potentiated by VWF. Collagen exposure and thrombin produced at the site of injury cause the adherent platelets to release their granule contents and also activate platelet prostaglandin synthesis leading to the formation of thromboxane A_2. Released ADP causes platelets to swell and aggregate. Additional platelets from the circulating blood are drawn to the area of injury. This continuing platelet aggregation promotes the growth of the haemostatic plug which soon covers the exposed connective tissue. The unstable primary haemostatic plug produced by these platelet reactions in the first minute or so following injury is usually sufficient to provide temporary control of bleeding. It seems likely that prostacyclin, produced by endothelial and smooth muscle cells in the vessel wall adjacent to the area of damage, is important in limiting the extent of the initial platelet plug.

Stabilization of the platelet plug by fibrin

Definitive haemostasis is achieved when fibrin formed by blood coagulation is added to the platelet mass and by platelet-induced clot retraction/compaction.

Following vascular injury, the formation of extrinsic Xase (VIIa, TF, PL and Ca^{2+}) initiates the coagulation cascade. Platelet aggregation and release reactions accelerate the coagulation process by providing abundant membrane phospholipid. Thrombin generated at the injury site converts soluble plasma fibrinogen into fibrin, potentiates platelet aggregation and secretion and also activates factor XI and XIII and cofactors V and VIII. The fibrin component of the haemostatic plug increases as the fused platelets autolyse and after a few hours the entire haemostatic plug is transformed into a solid mass of cross-linked fibrin. Nevertheless, because of incorporation of plasminogen and tPA (p. 273), this plug begins to autodigest during the same time frame.

Tests of haemostatic function

Defective haemostasis with abnormal bleeding may result from:

1 A vascular disorder;

2 Thrombocytopenia or a disorder of platelet function; or

3 Defective blood coagulation.

A number of simple tests are employed to assess the platelet, vessel wall and coagulation components of haemostasis.

Blood count and blood film examination

As thrombocytopenia is a common cause of abnormal bleeding, patients with suspected bleeding disorders should initially have a blood count including platelet count and blood film examination. In addition to establishing the presence of thrombocytopenia, the cause may be obvious (e.g. acute leukaemia).

Screening tests of blood coagulation

Screening tests provide an assessment of the 'extrinsic' and 'intrinsic' systems of blood coagulation and also the central conversion of fibrinogen to fibrin (Table 22.3).

The prothrombin time (PT) measures factors VII, X, V, prothrombin and fibrinogen. Tissue thromboplastin (a brain extract) and calcium are added to citrated plasma. The normal time for clotting is 10–14 s. It may be expressed as the international normalized ratio (INR) (p. 314).

The activated partial thromboplastin time (APTT) measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen. Three substances—phospholipid, a surface activator (e.g. kaolin) and calcium—are added to citrated plasma. The normal time for clotting is approximately 30–40 s.

Prolonged clotting times in the PT and APTT because of factor deficiency are corrected by the addition of normal plasma to the test plasma (50 : 50 mix). If there is no correction or incomplete

Table 22.3 Screening tests used in the diagnosis of coagulation disorders.

Screening tests	Abnormalities indicated by prolongation	Most common cause of disorder
Thrombin time (TT)	Deficiency or abnormality of fibrinogen or inhibition of thrombin by heparin or FDPs	Disseminated intravascular coagulation Heparin therapy
Prothrombin time (PT)	Deficiency or inhibition of one or more of the following coagulation factors: VII, X, V, II, fibrinogen	Liver disease Warfarin therapy DIC
Activated partial thromboplastin time (APTT or PTTK)	Deficiency or inhibition of one or more of the following coagulation factors: XII, XI, IX (Christmas disease), VIII (haemophilia), X, V, II, fibrinogen	Haemophilia, Christmas disease (+ conditions above)
Fibrinogen quantitation	Fibrinogen deficiency	Disseminated intravascular coagulation, liver disease

FDPs, fibrin degradation products.

NB. Platelet count and the tests of platelet function are also used in screening patients with a bleeding disorder.

correction with normal plasma, the presence of an inhibitor of coagulation is suspected.

The thrombin (clotting) time (TT) is sensitive to a deficiency of fibrinogen or inhibition of thrombin. Diluted bovine thrombin is added to citrated plasma at a concentration giving a clotting time of 14–16 s with normal subjects.

Specific assays of coagulation factors

Most factor assays are based on an APTT or PT in which all factors except the one to be measured are present in the substrate plasma. This usually requires a supply of plasma from patients with hereditary deficiency of the factor in question or artificially produced factor-deficient plasma. The corrective effect of the unknown plasma on the prolonged clotting time of the deficient substrate plasma is then compared with the corrective effect of normal plasma. Results are expressed as a percentage of normal activity.

A number of chemical, chromogenic and immunological methods are available for quantification of other proteins such as fibrinogen, VWF and factor VIII. Factor XIII activity can be assessed by testing for clot solubility in urea.

Bleeding time

The bleeding time is a useful test for abnormal platelet function including the diagnosis of VWF deficiency. It has largely been replaced by the platelet function analysis-100 (PFA-100) test (see below). It will be prolonged in thrombocytopenia but is normal in vascular causes of abnormal bleeding. The test involves the application of pressure to the upper arm with a blood pressure cuff, after which small incisions are made in the flexor surface forearm skin. Bleeding stops normally in 3–8 min.

Tests of platelet function

The most valuable investigation is platelet aggregometry which measures the fall in light absorbance in platelet-rich plasma as platelets aggregate. Initial (primary) aggregation is caused by an external agent, the secondary response to aggregating agents released from the platelets themselves. The five external aggregating agents most commonly used are ADP, collagen, ristocetin, arachidonic acid and adrenaline. The pattern of response to each agent helps to make the diagnosis (see Fig. 23.1). Flow cytometry is now increasingly used in routine practice to identify platelet glyoprotein defects.

In the PFA-100 test, citrated blood is aspirated through a capillary tube onto a membrane coated with collagen/ADP or collagen/adrenaline. Blood flow is maintained. Platelets begin to adhere and aggregate, primarily via VWF interactions with GPIb and GPIIb/IIIa, resulting in occlusion of the aperture.

The PFA-100 analysis may give false negative results with relatively common platelet defects. Full platelet function tests and VWF screening may be required to exclude abnormal platelet function, even if the PFA-100 test is normal.

Test of fibrinolysis

Increased levels of circulating plasminogen activator may be detected by demonstrating shortened euglobulin clot lysis times. A number of immunological methods are available to detect fibrinogen or fibrin degradation products (including D-dimers) in serum. In patients with enhanced fibrinolysis, low levels of circulating plasminogen may be detected.

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CHAPTER 23

23 Bleeding disorders caused by vascular and platelet abnormalities

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Abnormal bleeding may result from:

1 Vascular disorders;
2 Thrombocytopenia;
3 Defective platelet function; or
4 Defective coagulation.

The pattern of bleeding is relatively predictable depending on the aetiology. Vascular and platelet disorders tend to be associated with bleeding from mucous membranes and into the skin whereas in coagulation disorders the bleeding is often into joints or soft tissue (Table 23.1).

The first three categories are discussed in this chapter and the disorders of blood coagulation follow in Chapter 24.

Table 23.1 Clinical differences between diseases of platelets/vessel walls or coagulation factors.

Finding	Platelet/vessel walls	Coagulation
Mucosal bleeding	Common	Rare
Petechiae	Common	Rare
Deep haematomas	Rare	Characteristic
Bleeding from skin cuts	Persistent	Minimal
Sex of patient	Equal	>80% male

Vascular bleeding disorders

The vascular disorders are a heterogeneous group of conditions characterized by easy bruising and spontaneous bleeding from the small vessels. The underlying abnormality is either in the vessels themselves or in the perivascular connective tissues. Most cases of bleeding caused by vascular defects alone are not severe. Frequently, the bleeding is mainly in the skin causing petechiae, ecchymoses or both (Fig. 23.1). In some disorders there is also bleeding from mucous membranes. In these conditions the standard screening tests are normal. The bleeding time is normal and the other tests of haemostasis are also normal. Vascular defects may be inherited or acquired.

Inherited vascular disorders

Hereditary haemorrhagic telangiectasia

In this uncommon disorder, which is transmitted as an autosomal dominant trait. There are dilated microvascular swellings which appear during childhood and become more numerous in adult life. These telangiectasia develop in the skin, mucous membranes (Fig. 23.1a) and internal organs. Pulmonary and cerebral arteriovenous malformations are seen in a minority of cases. Recurrent epistaxes are frequent and recurrent gastrointestinal tract haemorrhage

Fig. 23.1 (a) Hereditary haemorrhagic telangiectasia: the characteristic small vascular lesions are obvious on the lips and tongue. (b) Senile purpura. (c) Characteristic perifollicular petechiae in vitamin C deficiency (scurvy).

may cause chronic iron deficiency anaemia. Treatment is with embolization, laser treatment, oestrogens, tranexamic acid and iron supplementation.

Connective tissue disorders

In the Ehlers–Danlos syndrome there are hereditary collagen abnormalities with purpura resulting from defective platelet aggregation, hyperextensibility of joints and hyperelastic friable skin. Pseudoxanthoma elasticum is associated with arterial haemorrhage and thrombosis. Mild cases may present with superficial bruising and purpura following minor trauma.

Giant cavernous haemangioma

These congenital malformations occasionally cause chronic activation of coagulation leading to laboratory features of disseminated intravascular coagulation (DIC) and in some cases thrombocytopenia.

Acquired vascular defects

1 Simple easy bruising is a common benign disorder which occurs in otherwise healthy women, especially those of child-bearing age.

2 Senile purpura caused by atrophy of the supporting tissues of cutaneous blood vessels is seen mainly on dorsal aspects of the forearms and hands (Fig. 23.1b).

3 Purpura associated with infections. Many bacterial, viral or rickettsial infections may cause purpura from vascular damage by the organism or as a result of immune complex formation (e.g. measles, dengue fever or meningococcal septicaemia).

4 The Henoch–Schönlein syndrome is usually seen in children and often follows an acute upper respiratory tract infection. It is an immunoglobulin A (IgA)-mediated vasculitis. The characteristic purpuric rash accompanied by localized oedema and itching is usually most prominent on the buttocks and extensor surfaces of the lower legs and elbows (Fig. 23.2). Painful joint swelling, haematuria and abdominal pain may also occur. It is usually a self-limiting condition but occasional patients develop renal failure.

(a) (b)

Fig. 23.2 Henoch–Schönlein purpura: (a) unusually severe purpura on legs with bullous formation in a 6-year-old child; and (b) early urticarial lesions.

5 Scurvy. In vitamin C deficiency, defective collagen may cause perifollicular petechiae, bruising and mucosal haemorrhage (Fig. 23.1c).
6 Steroid purpura. The purpura, which is associated with long-term steroid therapy or Cushing's syndrome, is caused by defective vascular supportive tissue.

Tranexamic acid and aminocaproic acid are useful antifibrinolytic drugs that may reduce bleeding resulting from vascular disorders or thrombocytopenia but are contraindicated in the presence of haematuria because they might lead to clots obstructing the renal tract.

Thrombocytopenia

Abnormal bleeding associated with thrombocytopenia or abnormal platelet function is characterized by spontaneous skin purpura (Fig. 23.3) and mucosal haemorrhage and prolonged bleeding after

(a) (b)

Fig. 23.3 (a) Typical purpura; and (b) massive subcutaneous haemorrhage in a patient with drug-induced thrombocytopenia.

Table 23.2 Causes of thrombocytopenia.

Failure of platelet production	
Selective megakaryocyte depression	
rare congenital defects	
drugs, chemicals, viral infections	
Part of general bone marrow failure	
cytotoxic drugs	
radiotherapy	
aplastic anaemia	
leukaemia	
myelodysplastic syndromes	
myelofibrosis	
marrow infiltration (e.g. carcinoma, lymphoma)	
multiple myeloma	
megaloblastic anaemia	
HIV infection	
Increased consumption of platelets	
Immune	
autoimmune	
idiopathic	
associated with systemic lupus erythematosus, chronic lymphocytic leukaemia or lymphoma	
infections: HIV, other viruses, malaria	
drug-induced	
heparin	
post-transfusional purpura	
feto-maternal alloimmune thrombocytopenia	
Disseminated intravascular coagulation	
Thrombotic thrombocytopenic purpura	
Abnormal distribution of platelets	
Splenomegaly	
Dilutional loss	
Massive transfusion of stored blood to bleeding patients	

HIV, human immunodeficiency virus.

trauma (Table 23.1). The main causes of thrombocytopenia are listed in Tables 23.2 and 23.3.

Failure of platelet production

This is the most common cause of thrombocytopenia and is usually part of a generalized bone marrow failure (Table 23.2). Selective megakaryocyte depression may result from drug toxicity or viral infection. Rarely, it is congenital as a result of mutation of the c-MPL thrombopoietin receptor, in association with absent radii, or in May–Hegglin anomaly, with large inclusions in granulocytes, or

Table 23.3 Thrombocytopenia as a result of drugs or toxins.

Bone marrow suppression	
Predictable (dose-related)	
ionizing radiation, cytotoxic drugs, ethanol	
Occasional	
chloramphenicol, co-trimoxazole, idoxuridine, penicillamine, organic arsenicals, benzene, etc.	
Immune mechanisms (proven or probable)	
Analgesics, anti-inflammatory drugs, gold salts	
Antimicrobials	
penicillins, sulphonamides, trimethoprim, para-aminosalicylate	
Sedatives, anticonvulsants	
diazepam, sodium valproate, carbamazepine	
Diuretics	
acetazolamide, chlorathiazides, frusemide	
Antidiabetics	
chlorpropamide, tolbutamide	
Others	
digitoxin, heparin, methyldopa, oxyprenolol, quinine, quinidine	
Platelet aggregation	
Ristocetin, heparin (p. 313)	

Wiskott–Aldrich syndrome (WAS) with eczema and immune deficiency. Diagnosis of these causes of thrombocytopenia is made from the clinical history, peripheral blood count, the blood film and bone marrow examination.

Increased destruction of platelets

Autoimmune (idiopathic) thrombocytopenic purpura

Autoimmune (idiopathic) thrombocytopenic purpura (ITP) may be divided into chronic and acute forms.

Chronic idiopathic thrombocytopenic purpura

This is a relatively common disorder. The highest incidence has been considered to be in women aged 15–50 years although some reports suggest an increasing incidence with age. It is the most common cause of thrombocytopenia without anaemia or neutropenia. It is usually idiopathic but may

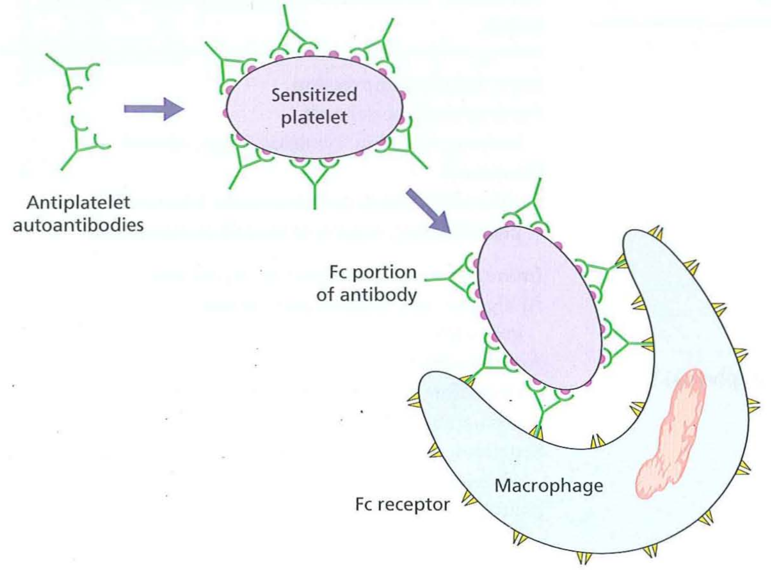


Fig. 23.4 The pathogenesis of thrombocytopenia in autoimmune thrombocytopenic purpura.

be seen in association with other diseases such as systemic lupus erythematosus (SLE), human immunodeficiency virus (HIV) infection, chronic lymphocytic leukaemia (CLL), Hodgkin's disease or autoimmune haemolytic anaemia (Table 23.2).

Pathogenesis

Platelet autoantibodies (usually IgG) result in the premature removal specific to platelets from the circulation by macrophages of the reticuloendothelial system, especially the spleen (Fig. 23.4). In many cases, the antibody is directed against antigen sites on the glycoprotein (GP) IIb–IIIa or Ib complex. The normal lifespan of a platelet is 7–10 days but in ITP this is reduced to a few hours. Total megakaryocyte mass and platelet turnover are increased in parallel to approximately five times normal.

Clinical features

The onset is often insidious with petechial haemorrhage, easy bruising and, in women, menorrhagia. Mucosal bleeding (e.g. epistaxes or gum bleeding) occurs in severe cases but fortunately intracranial haemorrhage is rare. The severity of bleeding in ITP is usually less than that seen in patients with comparable degrees of thrombocytopenia from bone marrow failure; this is attributed to the circulation of predominantly young, functionally superior platelets in ITP. Chronic ITP tends to relapse and remit spontaneously so the course may be difficult to predict. Many asymtomatic cases are discovered by a routine blood count.

The spleen is not palpable unless there is an associated disease causing splenomegaly.

Diagnosis

1 The platelet count is usually $10–50 \times 10^9/L$. The haemoglobin concentration and white cell count are typically normal unless there is iron deficiency anaemia because of blood loss.
2 The blood film shows reduced numbers of platelets, those present often being large.
3 The bone marrow shows normal or increased numbers of megakaryocytes.
4 Sensitive tests are able to demonstrate specific antiglycoprotein GPIIb/IIIa or GPIb antibodies on the platelet surface or in the serum in most patients. Platelet-associated IgG assays are less specific.

Treatment

As this is a chronic disease the aim of treatment should be to maintain a platelet count above the level at which spontaneous bruising or bleeding

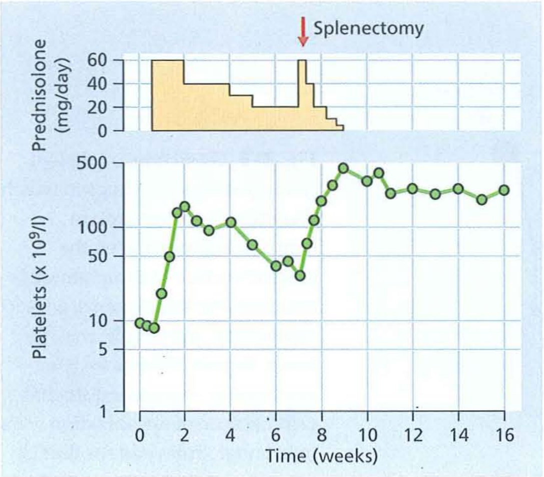


Fig. 23.5 Response to prednisolone in chronic immune thrombocytopenic purpura with subsequent relapse and response to splenectomy.

occurs with the minimum of intervention. In general, a platelet count above 50×10^9/L does not require treatment.

1 *Corticosteroids* Eighty per cent of patients remit on high-dose corticosteroid therapy. Prednisolone 1 mg/kg/day is the usual initial therapy in adults and the dosage is gradually reduced after 10–14 days. In poor responders the dosage is reduced more slowly but splenectomy or alternative immunosuppression is considered.

2 *Splenectomy* (Fig. 23.5) This operation is recommended in patients who have symptoms and still have platelets $<30 \times 10^9$/L after 3 months of steroid therapy or who require unacceptably high doses of steroids to maintain a platelet count above 30×10^9/L. Good results occur in most of the patients, but in patients with ITP refractory to steroids or immunoglobulin there may be little benefit. Splenunculi must be removed otherwise subsequent relapse of ITP can occur.

3 *High-dose intravenous immunoglobulin therapy* is able to produce a rapid rise in platelet count in the majority of patients. A regimen of 400 mg/kg/day for 5 days or 1 g/kg/day for 2 days is recommended. It is particularly useful in patients with life-threatening haemorrhage, in steroid-refractory ITP, during pregnancy or prior to surgery. The mechanism of action may be blockage of Fc receptors on macrophages or modification of autoantibody production.

4 *Immunosuppressive drugs* (e.g. vincristine, cyclophosphamide, azathioprine, mycophenolate mofetil or ciclosporin alone or in combination) are usually reserved for those patients who do not respond sufficiently to steroids and splenectomy.

5 *Monoclonal antibody* Rituximab (anti-CD20) produces responses in approximately 50%, which are often durable.

6 *Other treatments* that may elicit a remission include danazol (an androgen which may virilize in women) and intravenous anti-D immunoglobulin. It is often necessary to combine two drugs (e.g. danazol and an immunosuppressive agent).

7 *Platelet transfusions* Platelet concentrates are beneficial in patients with acute life-threatening bleeding. Their benefit will only last a few hours.

8 *Stem cell transplatation* has cured some severe cases.

Acute idiopathic thrombocytopenic purpura

This is most common in children. In approximately 75% of patients the episode follows vaccination or an infection such as chickenpox or infectious mononucleosis. Most cases are caused by non-specific immune complex attachments. Spontaneous remissions are usual but in 5–10% of cases the disease becomes chronic (lasting >6 months). Fortunately, morbidity and mortality in acute ITP is very low.

The diagnosis is one of exclusion and there is debate as to the need for bone marrow aspiration. If the platelet count is over 30×10^9/L no treatment is necessary unless the bleeding is severe. Those with counts below 20×10^9/L may be treated with steroids and/or intravenous immunoglobulin, especially if there is significant bleeding.

Infections

It seems likely that the thrombocytopenia associated with many viral and protozoal infections is immune-mediated. In HIV infection, reduced platelet production is also involved (p. 328).

Post-transfusion purpura

Thrombocytopenia occurring approximately 10 days after a blood transfusion has been attributed to antibodies in the recipient developing against the human

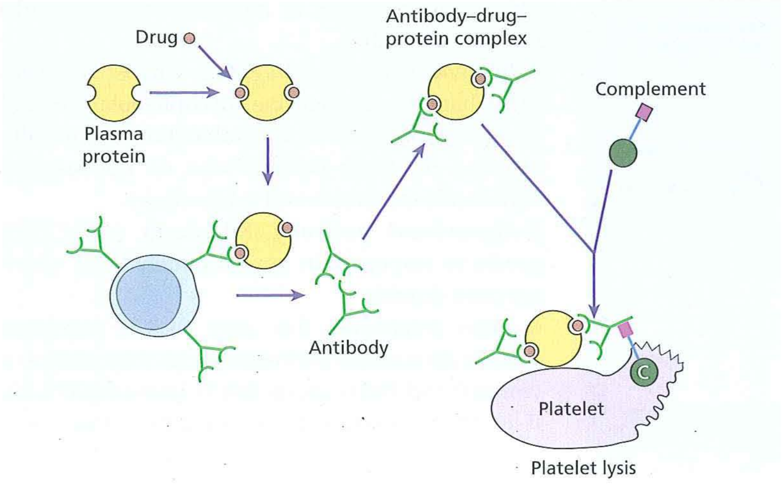


Fig. 23.6 Usual type of platelet damage caused by drugs in which an antibody–drug–protein complex is deposited on the platelet surface. If complement is attached and the sequence goes to completion, the platelet may be lysed directly. Otherwise it is removed by reticuloendothelial cells because of opsonization with immunoglobulin and/or the C3 component of complement.

platelet antigen-1a (HPA-1a) (absent from the patient's own platelets) on transfused platelets. The reason why the patient's own platelets are then destroyed is unknown. Treatment is with intravenous immunoglobulin, plasma exchange or corticosteroids.

Drug-induced immune thrombocytopenia

An immunological mechanism has been demonstrated as the cause of many drug-induced thrombocytopenias (Fig. 23.6). Quinine (including that in tonic water), quinidine and heparin are particularly common causes (Table 23.3).

The platelet count is often less than $10 \times 10^9/L$, and the bone marrow shows normal or increased numbers of megakaryocytes. Drug-dependent antibodies against platelets may be demonstrated in the sera of some patients. The immediate treatment is to stop all suspected drugs but platelet concentrates should be given to patients with dangerous bleeding.

Thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome

Thrombotic thrombocytopenic purpura (TTP) occurs in familial or acquired forms. There is deficiency of a ADAMTS13 metalloprotease which breaks down ultra large von Willebrand factor multimers (ULVWF) (Fig. 23.7). In the familial forms more than 50 ADAMTS13 mutations have been reported whereas in acquired forms it follows the development of an inhibitory IgG autoantibody, the presence of which may be stimulated by infection, autoimmune/connective tissue disease, certain drugs, stem cell transplantation or cardiac surgery. ULVWF multimeric strings secreted from Weibel–Palade bodies are anchored to the endothelial cells, and passing platelets adhere via their GPIbα receptors. Increasing platelet aggregation onto the ULVWF multimeric strings has the potential to form large, occlusive, platelet thrombi. These strings are capable of emobolising to microvessels downstream contributing to organ ischemia (Fig. 23.8). In the closely related haemolytic uraemic syndrome (HUS) ADAMTS13 levels are normal.

TTP has traditionally been described as pentad of thrombocytopenia, microangiopathic hamolytic anaemia, neurologic abnormalities, renal failure and fever. The microvascular thrombosis causes variable degrees of tissue ischaemia and infarction and is responsible for the microangiopathic haemolytic anaemia and thrombocytopenia. In current clinical practice, thombocytopenia, schistocytosis, and an impressively elevated serum lactate dehydrogenase (LDH) value are sufficient to suggest the diagnosis. The serum LDH is derived both from ischaemic or necrotic tissue cells and lysed red cells. Coagulation tests are normal in contrast to the findings in DIC (Fig. 24.8). The serum LDH is raised. ADAMTS13 is absent or severly reduced in plasma. Treatment is with plasma exchange, using fresh frozen plasma (FFP) or cryosupernatant. This removes the large molecular weight VWF multimers and the antibody and provides ADAMTS13.

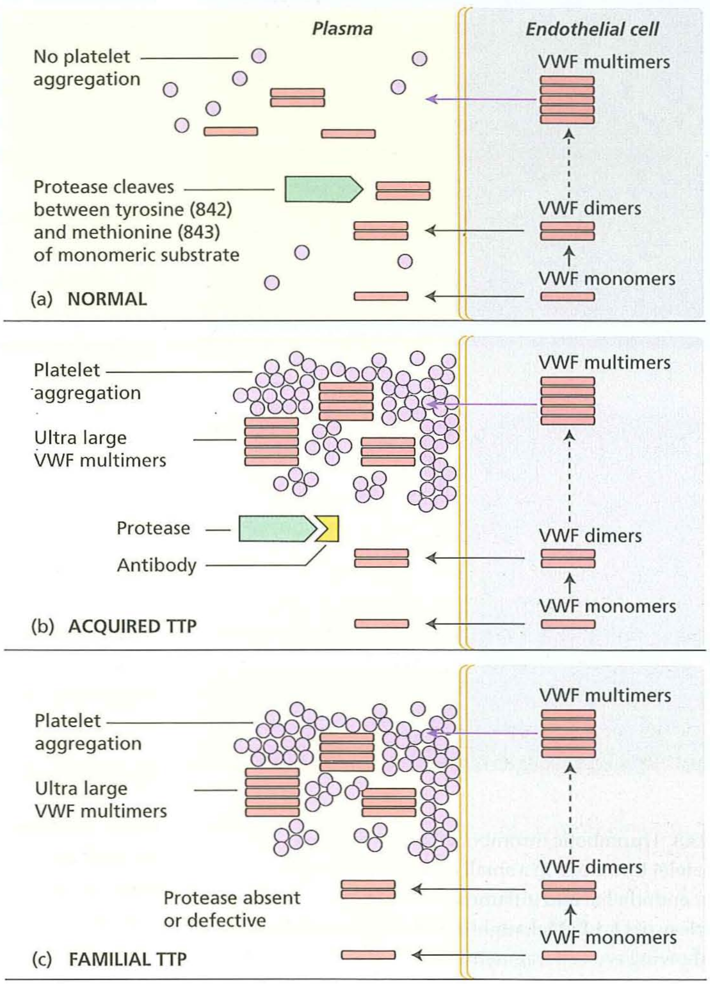


Fig. 23.7 Proposed pathogenesis of thrombotic thrombocytopenic purpura (TTP). Von Willebrand factor (VWF) consists of a series of VWF multimers each of molecular weight (MW) 250 kDa which are covalently linked. **(a)** Under physiological circumstances a metalloprotease ADAMTS13 cleaves high molecular weight multimers at a Tyr-842–Met-843 bond and the resulting VWF has an MW of 500–20 000 kDa. **(b)** In non-familial TTP, an antibody develops to the metalloprotease and so blocks cleavage of VWF multimers. **(c)** In congenital forms of TTP, the protease appears to be absent. In both cases, the resultant ultra large VWF multimers can bind platelets under high shear stress conditions and lead to platelet aggregation.

The platelet count and serum LDH are useful for monitoring the response to treatment. More recently, rituximab (anti-CD20) has been found effective in therapy, used in conjunction with plasma infusions or with plasma exhange. In refractory cases and chronic relapsing cases, high-dose corticosteroids, vincristine, intravenous immunoglobulin, rituximab and immunosuppressive therapy with azathioprine or cyclophosphamide have been used. In untreated cases mortality may approach 90%. Relapses are frequent.

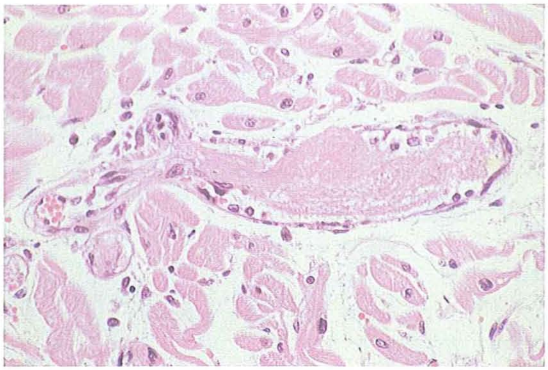
HUS in children has many common features but organ damage is limited to the kidneys. There is also usually diarrhoea. Fits are frequent. Many cases are associated with *Escherichia coli* infection with verotoxin 0157 or with other organisms, especially *Shigella*. Supportive renal dialysis and control of hypertension and fits are the mainstays of treatment. Platelet transfusions are contraindicated in HUS and TTP.

Disseminated intravascular coagulation

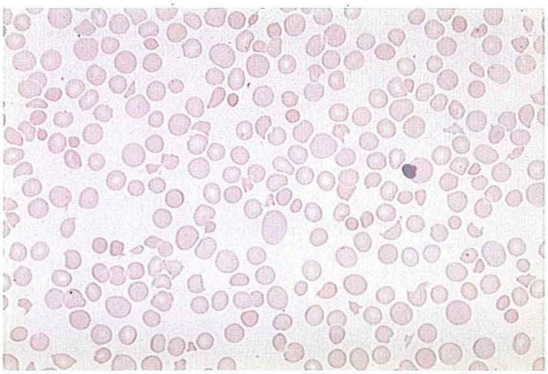
Thrombocytopenia may result from an increased rate of platelet destruction through consumption of platelets because of their participation in DIC (p. 298).

Increased splenic pooling

The major factor responsible for thrombocytopenia in splenomegaly is platelet 'pooling' by the spleen. In splenomegaly, up to 90% of platelets may be



(a)



(b)

Fig. 23.8 Thrombotic thrombocytopenic purpura. (a) Platelet thrombus in a small cardiac vessel with minor endothelial and inflammatory reaction. (Courtesy of Dr J.E. McLaughlin) (b) Peripheral blood film showing red cell fragmentation.

sequestered in the spleen whereas normally this accounts for approximately one-third of the total platelet mass (Fig. 23.9). Platelet lifespan is normal and in the absence of additional haemostatic defects, the thrombocytopenia of splenomegaly is not usually associated with bleeding.

Massive transfusion syndrome

Platelets are unstable in blood stored at 4°C and the platelet count rapidly falls in blood stored for more than 24 h. Patients transfused with massive amounts of stored blood (more than 10 units over a 24-h period) frequently show abnormal clotting and thrombocytopenia. These should be corrected by the use of platelet transfusions and FFP.

Disorders of platelet function

Disorders of platelet function are suspected in patients who show skin and mucosal haemorrhage and in whom the bleeding time is prolonged despite a normal platelet count. These disorders may be hereditary or acquired.

Hereditary disorders

Rare inherited disorders may produce defects at each of the different phases of the platelet reactions leading to the formation of the haemostatic platelet plug.

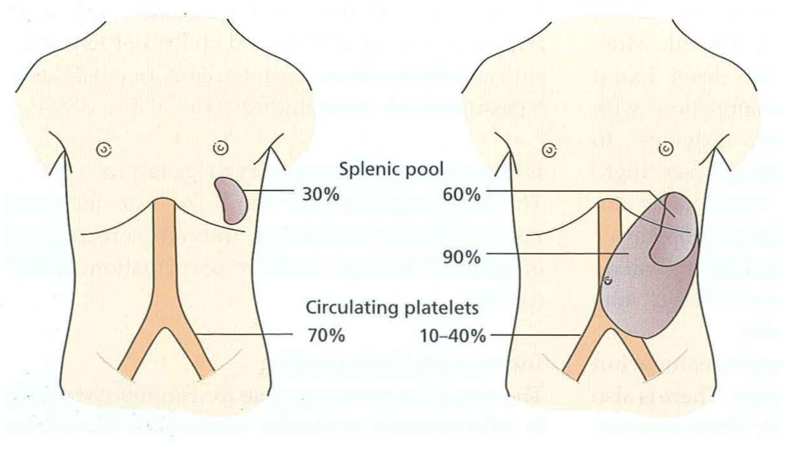


Fig. 23.9 The platelet distribution between the circulation and spleen in normal individuals (left), and in patients with moderate or massive splenomegaly (right).

Thrombasthenia (Glanzmann's disease)
This autosomal recessive disorder leads to failure of primary platelet aggregation because of a deficiency of membrane GPIIb (gene on chromosome 17). It usually presents in the neonatal period and, characteristically, platelets fail to aggregate *in vitro* to any agonist except ristocetin.

Bernard–Soulier syndrome
In this disease the platelets are larger than normal and there is a deficiency of GPIb (chromosome 23). There is defective binding to VWF, defective adherence to exposed subendothelial connective tissues and platelets do not aggregate with ristocetin. There is a variable degree of thrombocytopenia.

Storage pool diseases
In the rare grey platelet syndrome, the platelets are larger than normal and there is a virtual absence of α granules with deficiency of their proteins. In the more common β-storage pool disease there is a deficiency of dense granules.

Platelet function is abnormal in von Willebrand disease because of an inherited defect in VWF (p. 295).

Acquired disorders

Antiplatelet drugs
Aspirin therapy is the most common cause of defective platelet function. It produces an abnormal bleeding time and, although purpura may not be obvious, the defect may contribute to the associated gastrointestinal haemorrhage. The cause of the aspirin defect is inhibition of cyclo-oxygenase with impaired thromboxane A_2 synthesis (see Fig. 25.8). There is consequent impairment of the release reaction and aggregation with adrenaline and adenosine diphosphate (ADP). After a single dose the defect lasts 7–10 days, i.e. the life of the platelet. Aspirin is contraindicated in patients with gastrointestinal or genitourinary bleeding, retinal bleeding, peptic ulcer, haemophilia or severe hypertension. Dipyridamole inhibits platelet aggregation by blocking reuptake of adenosine and is usually used as an adjunct to oral anticoagulants. Clopidogrel inhibits binding of ADP to its platelet receptor and is mainly used for prevention of thrombotic events (e.g. after coronary stenting or angioplasty) in patients with a history of symptomatic atherosclerotic disease. Intravenous agents abciximab, eptifibatide and tirofiban are inhibitors of GPIIb/IIIa receptor sites and may be used in patients undergoing percutaneous coronary intervention with unstable angina and acute coronary syndromes. There is a risk of transient thrombocytopenia with these agents, especially with abciximab, and platelet transfusions may be needed.

Hyperglobulinaemia
Hyperglobulinaemia associated with multiple myeloma or Waldenström's disease may cause interference with platelet adherence, release and aggregation.

Myeloproliferative and myelodysplastic disorders
Intrinsic abnormalities of platelet function occur in many patients with essential thrombocythaemia and other myeloproliferative and myelodysplastic diseases and in paroxysmal nocturnal haemoglobinuria.

Uraemia
This is associated with various abnormalities of platelet function. Heparin, dextrans, alcohol and radiographic contrast agents may also cause defective function.

Diagnosis of platelet disorders

Patients with suspected platelet or blood vessel abnormalities should initially have a blood count and blood film examination (Fig. 23.10). Bone marrow examination is often needed in thrombocytopenic patients to determine whether or not there is a failure of platelet production. The marrow may also reveal one of the conditions associated with defective production (Table 23.2). In children and young adults with isolated thrombocytopenia, the marrow test is often not performed. In the elderly, the test is needed particularly to exclude myelodysplasia. In patients with thrombocytopenia, a negative drug history, normal or excessive numbers of marrow megakaryocytes and no other marrow abnormality or splenomegaly, ITP is the usual

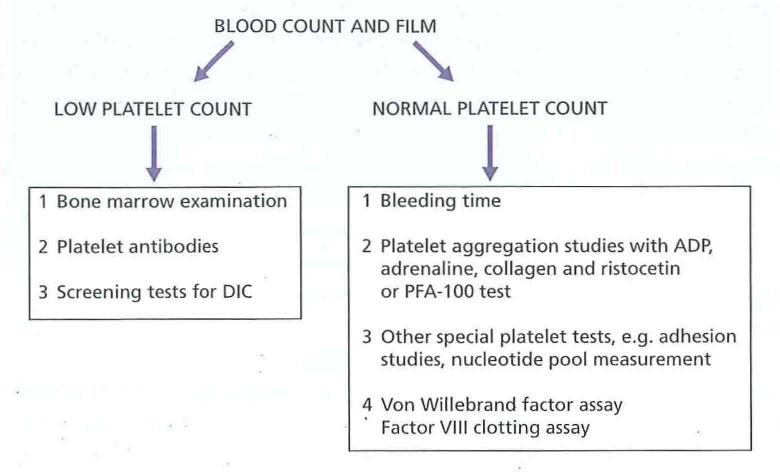


Fig. 23.10 Laboratory tests for platelet disorders. NB. Some intrinsic platelet functional disorders are associated with thrombocytopenia (e.g. Bernard–Soulier syndrome). ADP, adenosine diphosphate; DIC, disseminated intravascular coagulation.

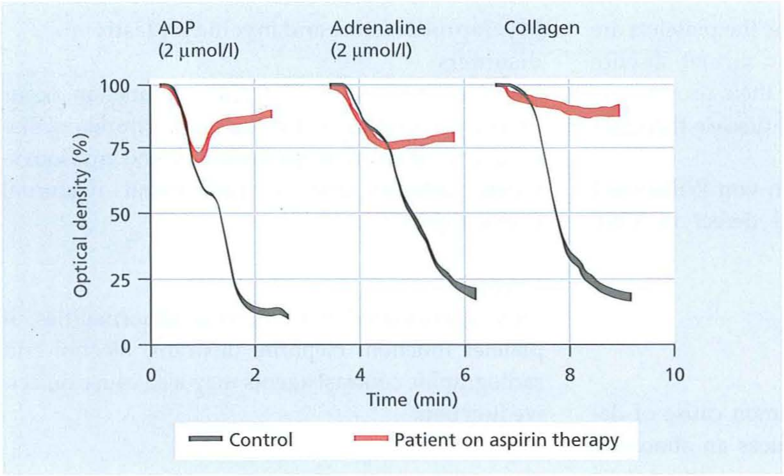


Fig. 23.11 Defective platelet aggregation in a patient on aspirin therapy. There is no secondary phase aggregation with adenosine diphosphate (ADP) and reduced responses to both adrenaline and collagen. Similar results are obtained in α-storage granule deficiency and cyclo-oxygenase deficiency.

diagnosis. Testing for platelet antibodies in serum or on the surface of platelets has not proved reliable in distinguishing ITP from other causes of thrombocytopenia. Screening tests for DIC are also useful, as are tests for an underlying disease (e.g. SLE or HIV infection).

When the blood count, including platelet count and blood film examination, are normal, a PFA-100 (platelet function analysis) or, much less frequently, a bleeding time is used to detect abnormal platelet function. In most patients with abnormal platelet function demonstrated by prolonged bleeding time, or the PFA-100 test, the defect is acquired and associated either with systemic disease (e.g. uraemia) or with aspirin therapy. The very rare hereditary defects of platelet function require more elaborate *in vitro* tests to define the specific abnormality. These include platelet aggregation studies (Fig. 23.11) and nucleotide pool measurements. If von Willebrand disease is suspected, assay of VWF and coagulation factor VIII are required.

Platelet transfusions

Transfusion of platelet concentrates are indicated in the following circumstances:

1 Thrombocytopenia or abnormal platelet function when bleeding or before invasive procedures and there is no alternative therapy available (e.g. steroids or high-dose immunoglobulin). The platelet count

should be above $50 \times 10^9/L$ before, for example, liver biopsy or lumbar puncture.

2 Prophylactically in patients with platelet counts of less than $5–10 \times 10^9/L$. If there is infection, potential bleeding sites or coagulopathy, the count should be kept above $20 \times 10^9/L$).

The indications for transfusion of platelet concentrates are discussed further on p. 349.

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CHAPTER 24

24 Coagulation disorders

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Hereditary coagulation disorders

Hereditary deficiencies of each of the coagulation factors have been described. Haemophilia A (factor VIII deficiency), haemophilia B (Christmas disease, factor IX deficiency) and von Willebrand disease (VWD) are the most common; the others are rare.

Haemophilia A

Haemophilia A is the most common of the hereditary clotting factor deficiencies. The prevalence is of the order of 30–100 per million population. The inheritance is sex-linked (Fig. 24.1) but up to 33% of patients have no family history and result from spontaneous mutation.

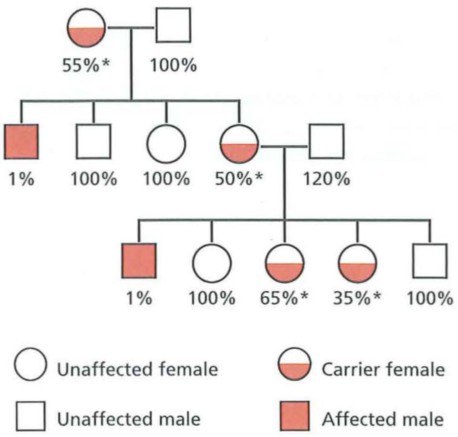

Fig. 24.1 A typical family tree in a family with haemophilia. Note the variable levels of factor VIII activity in carriers (*) because of random inactivation of X chromosome (Lyonization). The percentages show the degree of factor VIII activity as a percentage of normal.

Molecular genetics

The factor VIII gene is situated near the tip of the long arm of the X chromosome (Xq2.6 region). It is extremely large and consists of 26 exons. The factor VIII protein includes a triplicated region $A_1A_2A_3$ with 30% homology with each other, a duplicated homology region C_1C_2 and a heavy glycosylated B domain which is removed when factor VIII is activated by thrombin. The protein is synthesized in the liver and spleen.

The defect is an absence or low level of plasma factor VIII. Approximately half of the patients have missense or frameshift mutations or deletions in the factor VIII gene. In others a characteristic 'flip-tip' inversion is seen in which the factor VIII gene is broken by an inversion at the end of the X chromosome (Fig. 24.2). This mutation leads to a severe clinical form of haemophilia A.

Clinical features

Infants may suffer from profuse post-circumcision haemorrhage or develop joint and soft tissue bleeds

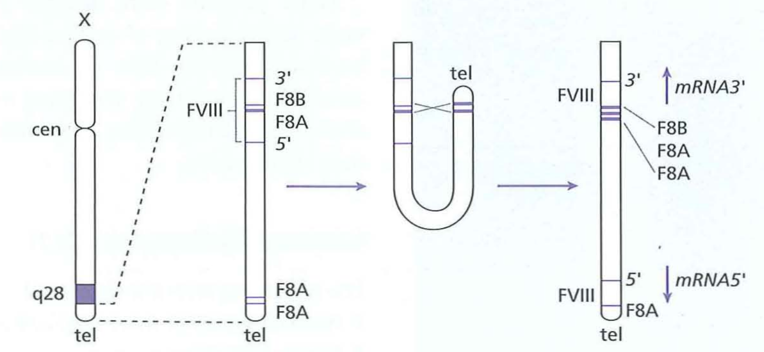

Fig. 24.2 The mechanism of the flip-tip inversion leading to disruption of the factor VIII gene. (Left) The orientation of the factor VIII gene is shown with the three copies of gene A in this region in (one within in intron 22 and two near the telomere). (Middle) During spermatogenesis at meiosis, the single X pairs with the Y chromosome in the homologous regions. The X chromosome is longer than the Y and there is nothing to pair with most of the long arm of X. The chromosome undergoes homologous recombination between the A genes. (Right) The final result is that the factor VIII gene is disrupted. cen, centromeric end; tel, telomere; the arrows indicate the direction of transcription from the A gene.

Fig. 24.3 Haemophilia A: acute haemarthrosis of the left knee joint with swelling of the suprapatellar region. There is wasting of the quadriceps muscles, particularly on the right.

and excessive bruising when they start to be active. Recurrent painful haemarthroses and muscle haematomas dominate the clinical course of severely affected patients and if poorly treated may lead to progressive joint deformity and disability (Figs 24.3–24.6). Local pressure can cause entrapment neuropathy or ischaemic necrosis. Prolonged bleeding occurs after dental extractions. Spontaneous haematuria and gastrointestinal haemorrhage, sometimes with obstruction resulting from intramucosal bleeding, can also occur. The clinical severity of the disease correlates with the extent of the factor VIII deficiency (Table 24.1). Operative and post-traumatic haemorrhage are life-threatening both in severely and mildly affected patients. Although not common, spontaneous intracerebral haemorrhage occurs more frequently than in the general population and is an important cause of death in patients with severe disease.

Haemophilic pseudotumours are large encapsulated haematomas with progressive cystic swelling from repeated haemorrhage. They may occur in fascial and muscle planes, large muscle groups and in the long bones, pelvis and cranium. These result from repeated subperiosteal haemorrhages with bone destruction and new bone formation.

As a result of human immunodeficiency virus (HIV) present in concentrates made from human plasma during the early 1980s, over 50% of haemophiliacs treated in the USA or Western Europe became infected with HIV. Acquired immune deficiency syndrome (AIDS) has been a common cause of death in severe haemophilia. Thrombocytopenia from HIV infection may exacerbate bleeding episodes.

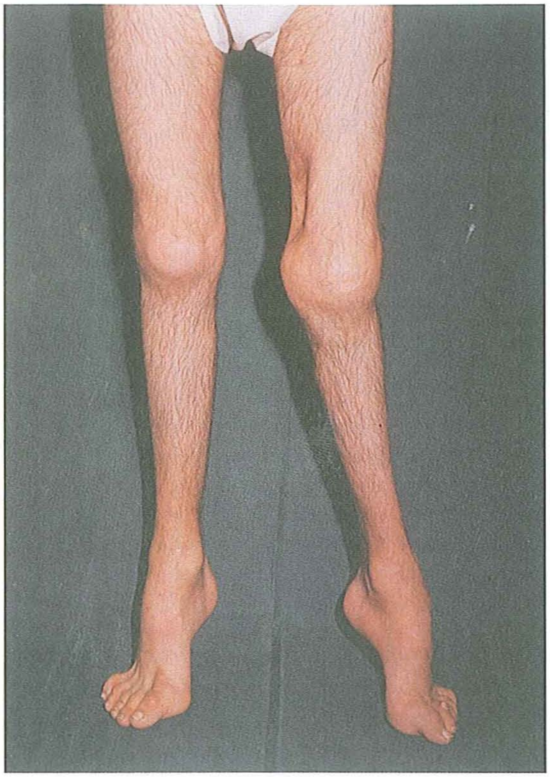

Fig. 24.4 Haemophilia A showing severe disability. The left knee is swollen with posterior subluxation of the tibia on the femur. The ankles and feet show residual deformities of talipes equinus, with some cavus and associated toe clawing. There is generalized muscle wasting. The scar on the medial side of the left lower thigh is the site of a previously excised pseudotumour.

Many patients were infected with hepatitis C virus before testing of donors and blood products became possible. This is causing an increasing amount of morbidity including chronic hepatitis, cirrhosis and hepatoma. Hepatitis B transmission may also be a risk.

Laboratory findings (Table 24.2)

The following tests are abnormal:

1 Activated partial thromboplastin time (APTT).
2 Factor VIII clotting assay.

The bleeding time and prothrombin time (PT) tests are normal.

Carrier detection and antenatal diagnosis

Until recently, carrier detection and antenatal diagnosis were limited to measuring plasma levels of factor VIII and von Willebrand factor (VWF). Carriers are now better detected with DNA probes. A known specific mutation can be identified or restriction fragment length polymorphisms within or close to the factor VIII gene allows the mutant allele to be tracked. Chorionic biopsies at 8–10 weeks' gestation provide sufficient fetal DNA for analysis. Antenatal diagnosis is also possible following the demonstration of low levels of factor VIII in fetal blood obtained at 16–20 weeks' gestation from the umbilical vein by ultrasound-guided needle aspiration.

Fig. 24.5 Haemophilia A: massive haemorrhage in the area of the right buttock.

Fig. 24.6 Haemophilia A: radiographic appearances of the right elbow joint in a 25-year-old male. The joint space has been destroyed and there is bony ankylosis. Subchondral cystic areas are prominent.

Treatment

Most patients attend specialized haemophilia centres where there is a multidisciplinary team dedicated to their care. Bleeding episodes are treated with factor VIII replacement therapy and spontaneous bleeding is usually controlled if the patient's factor VIII level is raised to 30–50% of normal. Guidelines exist for the plasma level to be achieved for different types of haemorrhage. For major surgery, serious post-traumatic bleeding or when haemorrhage is occurring at a dangerous site, the factor VIII level should be elevated to 100% and then maintained above 50% when acute bleeding has stopped, until healing has occurred. On average, factor VIII infusion produces a plasma increment of 2 U/dL per unit infused per kilogram body weight. Roughly, the dose to be infused (units) = (weight (kg) × increment needed (U/dL))/2.

Recombinant factor VIII (five different commercial preparations) and immunoaffinity-purified factor VIII preparations, which are heat or solvent-detergent treated, are now available for clinical use and virtually eliminate the risk of viral transmission.

DDAVP (desmopressin) provides an alternative means of increasing the plasma factor VIII level in milder haemophiliacs. Following the intravenous administration of this drug, there is a 2–4-fold rise maximum at 30–60 min in the patient's own factor VIII by release from endothelial cells and this rise is proportional to the resting level. DDAVP may also be taken subcutaneously or nasally—this has been used as immediate treatment for mild haemophilia after accidental trauma or haemorrhage. DDAVP has an antidiuretic action and should be avoidedin the elderly; fluid restitution is advised after its use.

Local supportive measures used in treating haemarthroses and haematomas include resting the affected part and the prevention of further trauma.

Table 24.1 Correlation of coagulation factor activity and disease severity in haemophilia A or B.

Coagulation factor activity (percentage of normal)	Clinical manifestations
<2	Severe disease Frequent spontaneous bleeding into joints, muscles, internal organs from early life Joint deformity and crippling if not adequately treated
2–10	Moderate disease Bleeding after minor trauma Occasional spontaneous episodes
>10–30	Mild disease Minor bleeding after significant trauma, surgery

Table 24.2 Main clinical and laboratory findings in haemophilia A, factor IX deficiency (haemophilia B, Christmas disease) and von Willebrand disease.

	Haemophilia A	Factor IX deficiency	von Willebrand disease
Inheritance	Sex-linked	Sex-linked	Dominant (incomplete)
Main sites of haemorrhage	Muscle, joints, post-trauma or postoperative	Muscle, joints, post-trauma or postoperative	Mucous membranes, skin cuts, post-trauma or postoperative
Platelet count	Normal	Normal	Normal
Bleeding time	Normal	Normal	Prolonged
Prothrombin time	Normal	Normal	Normal
Partial thromboplastin time	Prolonged	Prolonged	Prolonged or normal
Factor VIII	Low	Normal	May be moderately reduced
Factor IX	Normal	Low	Normal
VWF	Normal	Normal	Low or abnormal function (Table 24.3)
Ristocetin-induced platelet aggregation	Normal	Normal	Impaired

VWF, von Willebrand factor.

Prophylactic treatment

The increased availability of factor VIII concentrates that may be stored in domestic refrigerators has dramatically altered haemophilia treatment. At the earliest suggestion of bleeding, the haemophilic child may be treated at home. This advance has reduced the occurrence of crippling haemarthroses and the need for inpatient care. Severely affected patients are now reaching adult life with little or no arthritis. After the first spontaneous joint bleed, most boys with severe haemophilia are started on prophylactic factor VIII three times a week, aiming to keep their factor VIII levels above 1%. This may require the placement of a vascular access device such as Port-a-Cath if venous access is difficult.

Haemophiliacs are advised to have regular conservative dental care. Haemophilic children and their parents often require extensive help with social and psychological matters. With modern treatment the lifestyle of a haemophilic child can be almost normal but certain activities such as body contact sports are to be avoided.

Gene therapy

Because it is only necessary to maintain factor levels >1% to prevent most of the mortality and morbidity of factor VIII or IX deficiency, there is great interest in gene-based therapy. Various viral vectors (retroviral, adeno-associated) as well as non-viral vectors are being explored. Phase 1 trials have been carried out for both haemophilia A and B.

Inhibitors

One of the most serious complications of haemophilia is the development of antibodies (inhibitors) to infused factor VIII which occurs in 5–10% of (mainly severe) patients, especially children. This renders the patient refractory to further replacement therapy so that tremendous doses have to be given to achieve a significant rise in plasma factor VIII activity. Immunosuppression and immune tolerance regimes have been used in an attempt to eradicate the antibody. Recombinant activated factor VII (VIIa) and activated prothrombin complex concentrates (FEIBA—factor VIII inhibitor bypassing activity) can be useful in the treatment of bleeding episodes.

Factor VIIa complexes with tissue factor exposed at the site of injury and produces local haemostasis. The process is independent of factor VIII or IX and is not affected by their inhibitors. Factor VIIa has a short half-life and therefore frequent doses may be needed. In the longer term, immunosuppression with cyclophosphamide, intravenous immunoglobulin and high-dose factor VIII has also been successful.

Factor IX deficiency

The inheritance and clinical features of factor IX deficiency (Christmas disease, haemophilia B) are identical to those of haemophilia A. Indeed, the two disorders can only be distinguished by specific coagulation factor assays. The incidence is one-fifth that of haemophilia A. Factor IX is coded by a gene close to the gene for factor VIII near the tip of the long arm of the X chromosome. Its synthesis, like that of prothrombin, factor VII, factor X and protein C, is vitamin K-dependent. Carrier detection and antenatal diagnosis is performed as for haemophilia A. The principles of replacement therapy are similar to those of haemophilia A. Bleeding episodes are treated with high-purity factor IX concentrates. Because of its longer biological half-life, infusions do not have to be given as frequently as factor VIII concentrates in haemophilia A. Recombinant factor IX is available. Higher doses are needed compared with plasma-derived factor IX.

Laboratory findings (Table 24.2)

The following tests are abnormal:

1 APTT

2 Factor IX clotting assay

As in haemophilia A, the bleeding time and PT tests are normal.

Von Willebrand disease

In this disorder there is either a reduced level or abnormal function of VWF resulting from a point mutation or major deletion. VWF is produced in endothelial cells and megakaryocytes. It has two roles (Chapter 22). It promotes platelet adhesion to damaged endothelium and it is the carrier molecule for factor VIII, protecting it from premature destruction. The latter property explains the occasional reduced factor VIII levels found in VWD.

Chronic elevation of VWF is part of the acute phase response to injury, inflammation, neoplasia or pregnancy. VWF is synthesized as a large 300-kDa protein which then forms multimers up to 10^6 Da in weight. Three types of VWD have been described (Table 24.3). Type 2 is divided into four subtypes depending on the type of functional defect. Type 1 accounts for 75% of cases.

VWD is the most common inherited bleeding disorder. Usually, the inheritance is autosomal dominant with varying expression. The severity of the bleeding is variable. Typically, there is mucous membrane bleeding (e.g. epistaxes, menorrhagia), excessive blood loss from superficial cuts and abrasions, and operative and post-traumatic haemorrhage. The severity is variable in the different types. Haemarthroses and muscle haematomas are rare, except in type 3 disease.

Laboratory findings (Table 24.2)

1 The bleeding time can be prolonged. This test is usually replaced by the PFA-100 test (see p. 277).

2 Factor VIII levels are often low. If low, a factor VIII VWF binding assay is performed.

Table 24.3 Classification of von Willebrand disease.

Type 1	Quantitative partial deficiency
Type 2	Functional abnormality
Type 3	Complete deficiency

Secondary classification of type 2 VWD

Subtype	Platelet-associated function	Factor VIII binding capacity	High MW VWF multimers
2A	Decreased	Normal	Absent
2B	Increased affinity for GPIb	Normal	Usually reduced/absent
2M	Decreased	Normal	Normal or ultra large
2N	Normal	Reduced	Normal

GPIb, glycoprotein Ib; MW, molecular weight; VWD, von Willebrand disease; VWF, von Willebrand factor.

3 The APTT may be prolonged.
4 VWF levels are usually low.
5 There is defective platelet aggregation by patient plasma in the presence of ristocetin (VWF: Rco). Aggregation to other agents (adenosine diphosphate (ADP), thrombin or adrenaline) is usually normal.
6 Collagen-binding function (VWF: CB) is usually reduced.
7 Multimer analysis is useful for diagnosing different subtypes (Table 24.3).
8 The platelet count is normal except for type 2B disease (where it is low).

Treatment

Options are as follows:
1 Local measures and antifibrinolytic agent (e.g. tranexamic acid for mild bleeding).
2 DDAVP infusion for those with type 1 VWD. This releases VWF from endothelial stage sites 30 min after intravenous infusion.
3 High-purity factor VWF concentrates for patients with very low VWF levels. Factor VIII concentrate may also be given for more rapid correction.

Hereditary disorders of other coagulation factors

All these disorders (deficiency of fibrinogen, prothrombin, factors V, VII, combined V and VIII, factors X, XI, XIII) are rare. In most the inheritance is autosomal recessive. Factor XI deficiency is seen mainly in Ashkenazi Jews and occurs in either sex. The bleeding risk is not related to severity of the deficiency, and is absent from muscles and joints. The severity is poorly related to the factor XI level in plasma. It usually causes excess bleeding only after trauma such as surgery, and is treated by factor XI concentrate or fresh frozen plasma. Factor XIII deficiency produces a severe bleeding tendency, characteristically with umbilical stump bleeding. Specific concentrates or recombinant preparation of factors VII, XI and XIII are now available.

Acquired coagulation disorders

The acquired coagulation disorders (Table 24.4) are more common than the inherited disorders. Unlike the inherited disorders, multiple clotting factor deficiencies are usual.

Table 24.4 The acquired coagulation disorders.

Deficiency of vitamin K-dependent factors
Haemorrhagic disease of the newborn
Biliary obstruction
Malabsorption of vitamin K (e.g. tropical sprue, gluten-induced enteropathy)
Vitamin K-antagonist therapy (e.g. coumarins, indandiones)
Liver disease
Disseminated intravascular coagulation
Inhibition of coagulation
Specific inhibitors (e.g. antibodies against factor VIII)
Non-specific inhibitors (e.g. antibodies found in systemic lupus erythematosus, rheumatoid arthritis)
Miscellaneous
Diseases with M-protein production
L-Asparaginase
Therapy with heparin, defibrinating agents or thrombolytics
Massive transfusion syndrome

Vitamin K deficiency

Fat-soluble vitamin K is obtained from green vegetables and bacterial synthesis in the gut. Deficiency may present in the newborn (haemorrhagic disease of the newborn) or in later life.

Deficiency of vitamin K is caused by an inadequate diet, malabsorption or inhibition of vitamin K by drugs such as warfarin which act as vitamin K antagonists. Warfarin is associated with a decrease in the functional activity of factors II, VII, IX and X and proteins C and S, but immunological methods show normal levels of these factors. The non-functional proteins are called PIVKA (proteins formed in vitamin K absence). Conversion of PIVKA factors to their biologically active forms is a post-translational event involving carboxylation of glutamic acid residues in the N-terminal region where these factors show strong sequence homology (Fig. 24.7). Gamma-carboxylated glutamic acid binds calcium ions through which it forms

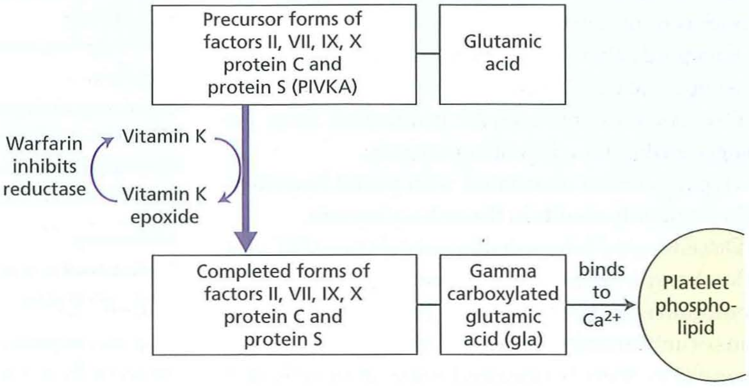

Fig. 24.7 The action of vitamin K in γ-carboxylation of glutamic acid in coagulation factors which are then able to bind Ca^{2+} and attach to the platelet phospholipid.

a complex with phospholipid. In the process of carboxylation, vitamin K is converted to vitamin K epoxide which is cycled back to the reduced form by reductases. Warfarin interferes with the reduction of vitamin K epoxide leading to a functional vitamin K deficiency.

Haemorrhagic disease of the newborn

Vitamin K-dependent factors are low at birth and fall further in breast-fed infants in the first few days of life. Liver cell immaturity, lack of gut bacterial synthesis of the vitamin and low quantities in breast milk may all contribute to a deficiency which may cause haemorrhage, usually on the second to fourth day of life, but occasionally during the first 2 months.

Diagnosis

The PT and APTT are both abnormal. The platelet count and fibrinogen are normal with absent fibrin degradation products.

Treatment

1 Prophylaxis. For many years vitamin K has been given to all newborn babies as a single intramuscular injection of 1 mg. This remains the most appropriate and safest treatment. Following epidemiological evidence suggesting a possible link between intramuscular vitamin K and an increased risk of childhood tumours (which has not been substantiated), some centres recommended an oral regimen but this is less effective in prevention and repeat doses are needed up to day 28.

2 In bleeding infants: vitamin K 1 mg intramuscularly is given every 6 h with, initially, fresh frozen plasma if haemorrhage is severe.

Vitamin K deficiency in children or adults

Deficiency resulting from obstructive jaundice, pancreatic or small bowel disease occasionally causes a bleeding diathesis in children or adults.

Diagnosis

Both PT and APTT are prolonged. There are low plasma levels of factors II, VII, IX and X.

Treatment

1 Prophylaxis: vitamin K 5 mg/day orally.

2 Active bleeding or prior to liver biopsy: vitamin K 10 mg slowly intravenously. Some correction of PT is usual within 6 h. The dose should be repeated on the next 2 days after which optimal correction is usual.

Liver disease

Multiple haemostatic abnormalities contribute to a bleeding tendency and may exacerbate haemorrhage from oesophageal varices.

1 Biliary obstruction results in impaired absorption of vitamin K and therefore decreased synthesis of factors II, VII, IX and X by liver parenchymal cells.

2 With severe hepatocellular disease, in addition to a deficiency of these factors, there are often reduced

levels of factor V and fibrinogen and increased amounts of plasminogen activator.

3 Functional abnormality of fibrinogen (dysfibrinogenaemia) is found in many patients.

4 Decreased thrombopoietin production from the liver contributes to thrombocytopenia.

5 Hypersplenism associated with portal hypertension frequently results in thrombocytopenia.

6 Disseminated intravascular coagulation (DIC; see below) may be related to release of thromboplastins from damaged liver cells and reduced concentrations of antithrombin, protein C and α_2-antiplasmin. In addition, there is impaired removal of activated clotting factors and increased fibrinolytic activity.

Disseminated intravascular coagulation

Widespread inappropriate intravascular deposition of fibrin with consumption of coagulation factors and platelets occurs as a consequence of many disorders which release procoagulant material into the circulation or cause widespread endothelial damage or platelet aggregation (Table 24.5). It may be associated with a fulminant haemorrhagic or thrombotic syndrome with organ dysfunction or run a less severe and more chronic course. The main clinical presentation is with bleeding but 5–10% of patients manifest microthrombotic lesions (e.g. with gangrene of limbs).

Pathogenesis (Fig. 24.8)

The key event underlying DIC is increased activity of tissue factor. This can come from its release into the circulation from damaged tissues present on tumour cells or from up-regulation of tissue factor on circulating monocytes or endothelial cells

Table 24.5 Causes of disseminated intravascular coagulation.

Infections
Gram-negative and meningococcal septicaemia
Clostridium welchii septicaemia
Severe Falciparum malaria
Viral infection—varicella, HIV, hepatitis, cytomegalovirus
Malignancy
Widespread mucin-secreting adenocarcinoma
Acute promyelocytic leukaemia
Obstetric complications
Amniotic fluid embolism
Premature separation of placenta
Eclampsia; retained placenta
Septic abortion
Hypersensitivity reactions
Anaphylaxis
Incompatible blood transfusion
Widespread tissue damage
Following surgery or trauma
After severe burns
Vascular abnormalities
Kasabach–Merritt syndrome
Leaking prosthetic valves
Cardiac bypass surgery
Vascular aneurysms
Miscellaneous
Liver failure
Pancreatitis
Snake and invertebrate venoms
Hypothermia
Heat stroke
Acute hypoxia
Massive blood loss

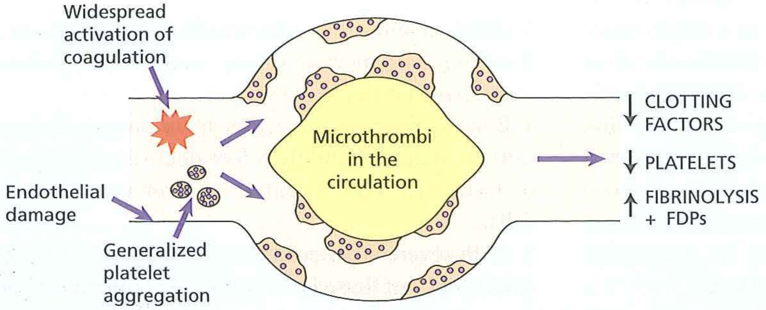

Fig. 24.8 The pathogenesis of disseminated intravascular coagulation and the changes in clotting factors, platelets and fibrin degradation products (FDPs) that occur in this syndrome.

in response to pro-inflammatory cytokines (e.g. interleukin-1, tumour necrosis factor, endotoxin).

1 DIC may be triggered by the entry of procoagulant material into the circulation in the following situations: severe trauma, amniotic fluid embolism, premature separation of the placenta, widespread mucin-secreting adenocarcinomas, acute promyelocytic leukaemia (AML type M_3), liver disease, severe falciparum malaria, haemolytic transfusion reaction and some snake bites.

2 DIC may also be initiated by widespread endothelial damage and collagen exposure (e.g. endotoxaemia, Gram-negative and meningococcal septicaemia, septic abortion), certain virus infections and severe burns or hypothermia.

In addition to its role in the deposition of fibrin in the microcirculation, intravascular thrombin formation produces large amounts of circulating fibrin monomers which form complexes with fibrinogen. Intense fibrinolysis is stimulated by thrombi on vascular walls and the release of split products interferes with fibrin polymerization, thus contributing to the coagulation defect. The combined action of thrombin and plasmin normally causes depletion of fibrinogen and all coagulation factors. Intravascular thrombin also causes widespread platelet aggregation and deposition in the vessels. The bleeding problems which may be a feature of DIC are compounded by thrombocytopenia caused by consumption of platelets.

Clinical features

These are dominated by bleeding, particularly from venepuncture sites or recent wounds (Fig. 24.9a). There may be generalized bleeding in the gastrointestinal tract, the oropharynx, into the lungs, urogenital tract and in obstetric cases vaginal bleeding may be particularly severe. Less frequently, microthrombi may cause skin lesions, renal failure, gangrene of the fingers or toes (Fig. 24.9b) or cerebral ischaemia.

Some patients may develop subacute or chronic DIC, especially with mucin-secreting adenocarcinoma. Compensation by the liver may render some of the coagulation tests normal.

Laboratory findings (Table 24.6)

In many acute syndromes the blood may fail to clot because of gross fibrinogen deficiency.

(a)

(b)

Fig. 24.9 Clinical features of disseminated intravascular coagulation: **(a)** indurated and confluent purpura of the arm; **(b)** peripheral gangrene with swelling and discoloration of the skin of the feet in fulminant disease.

Tests of haemostasis

1 The platelet count is low.
2 Fibrinogen concentration low.
3 The thrombin time is prolonged.
4 High levels of fibrin degradation products such as D-dimers are found in serum and urine.
5 The PT and APTT are prolonged in the acute syndromes.

Blood film examination

In many patients there is a haemolytic anaemia ('microangiopathic') and the red cells show prominent fragmentation because of damage caused when passing through fibrin strands in small vessels (p. 69).

Table 24.6 Haemostasis tests: typical results in acquired bleeding disorders.

	Platelet count	Prothrombin time	Activated partial thromboplastin time	Thrombin time
Liver disease	Low	Prolonged	Prolonged	Normal (rarely prolonged)
DIC	Low	Prolonged	Prolonged	Grossly prolonged
Massive transfusion	Low	Prolonged	Prolonged	Normal
Oral anticoagulants	Normal	Grossly prolonged	Prolonged	Normal
Heparin	Normal (rarely low)	Mildly prolonged	Prolonged	Prolonged
Circulating anticoagulant	Normal	Normal or prolonged	Prolonged	Normal

DIC, Disseminated intravascular coagulation.

Table 24.7 Indications for the use of fresh frozen plasma (National Institutes of Health Consensus Guidelines).

Coagulation factor deficiency (where specific or combined factor concentrate is not available)
Reversal of warfarin effect
Multiple coagulation defects (e.g. in patients with liver disease, DIC)
Massive blood transfusion with coagulopathy and clinical bleeding
Thrombotic thrombocytopenic purpura
Deficiencies of antithrombin*, protein C* or protein S
Some patients with immunodeficiency syndromes

DIC, disseminated intravascular coagulation.
* Antithrombin and protein C concentrates now available.

Treatment

Treatment of the underlying cause is most important. The management of patients who are bleeding differs from that of patients with thrombotic problems.

Bleeding

Supportive therapy with fresh frozen plasma (Table 24.7) and platelet concentrates is indicated in patients with dangerous or extensive bleeding. Cryoprecipitate provides a more concentrated source of fibrinogen and red cell transfusions may be required.

Thrombosis

The use of heparin or antiplatelet drugs to inhibit the coagulation process is considered in those with thrombotic problems such as dermal ischaemia. Fibrinolytic inhibitors should not be considered because failure to lyse thrombi in organs such as the kidney may have adverse effects. Antithrombin concentrates or recombinant human protein C may be used to inhibit DIC in severe cases with sepsis (e.g. meningococcal septicaemia). There is reduced activated protein C (APC) in severe sepsis and recombinant human APC has been found to reduce mortality in this setting.

Coagulation deficiency caused by antibodies

Circulating antibodies to coagulation factors are occasionally seen with an incidence of approximately 1 per millon per year. Alloantibodies to factor VIII occur in 5–10% of haemophiliacs. Factor VIII autoantibodies may also result in a bleeding syndrome. These immunoglobulin G (IgG) antibodies occur rarely post-partum, in certain immunological disorders (e.g. rheumatoid arthritis), in cancer and in old age. Treatment usually consists of a combination of immunosuppression and treatment with factor replacement, usually as human factor VIII, recombinant VIIa or activated prothrombin complex concentrate (FEIBA).

Another protein known as the lupus anticoagulant interferes with lipoprotein-dependent stages of coagulation and is usually detected by prolongation of the APTT test (Table 24.6). This inhibitor is detected in 10% of patients with systemic lupus erythematosus (SLE) and in patients with other autoimmune diseases who frequently have antibodies

to other lipid-containing antigens (e.g. cardiolipin). The antibody is not associated with a bleeding tendency but there is an increased risk of arterial or venous thrombosis and, as with other causes of thrombophilia, an association with recurrent miscarriage (Chapter 25).

Massive transfusion syndrome

Many factors may contribute to a bleeding disorder following massive transfusion. Blood loss results in reduced levels of platelets, coagulation factors and inhibitors. Further dilution of these factors occurs during replacement with red cells.

Management

Platelet concentrates are given to maintain a platelet count >50 × 10^9/L or 80–100 × 10^9/L in cerebral injury or polytrauma. The PT and APTT should be kept to less than 1.5 times normal with fresh frozen plasma given initially at 15 mL/kg. Cryoprecipitate is given to keep fibrinogen at least 1 g/L. Recombinant VIIa is increasingly used in patients with massive blood loss after trauma or surgery to reduce haemorrhage.

The results of haemostasis screening tests in acquired bleeding disorders are shown in Table 24.6 and a summary of the indications for use of fresh frozen plasma in Table 24.7.

Thromboelastography: near-patient testing

Thromboelastography (TEG) is a technique for a global assessment of haemostatic function of a single blood sample in which the reaction of platelets with the protein coagulation cascade is observed from the time of the initial platelet fibrin interaction

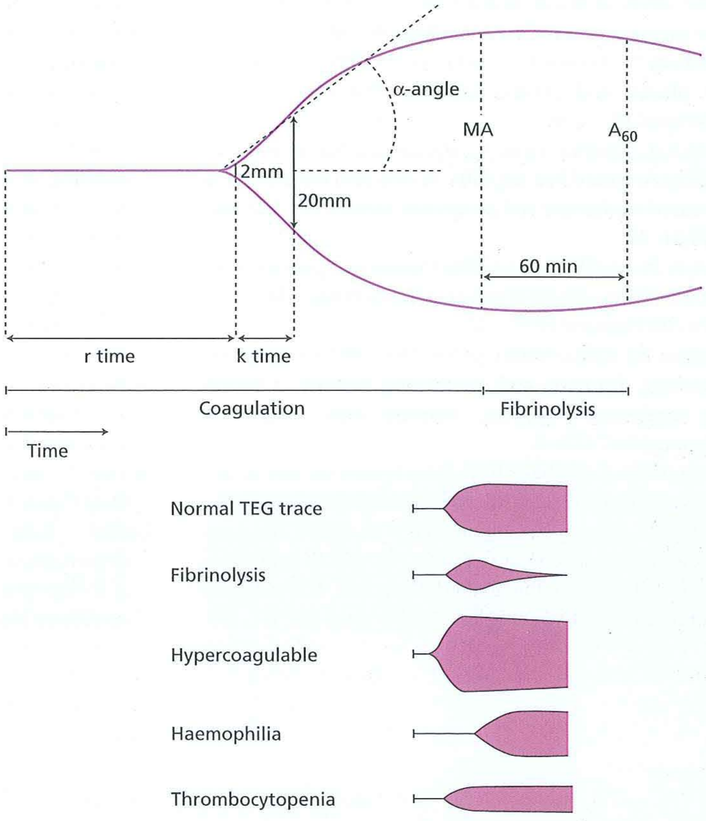

Fig. 24.10 Thromboelastography (TEG): normal trace and appearances in different pathological states. α-angle, speed of solid clot formation; A_{60}, measure of clot lysis or retraction at 60 min; k, clot formation time; MA, absolute strength of fibrin clot; r, rate of initial fibrin formation. (From S.V. Mallett and D.J.A. Cox (1992). Thromboelastography. *Br J Anaesth* **69**,307–13 with permission)

through platelet aggregation, clot strengthening and fibrin cross-linkage to eventual clot lysis. It is suited as a monitor of haemostasis in surgery (e.g. of the liver or heart) associated with haemostatic defects. Freshly drawn blood is placed in a cuvette which is oscillated, the motion being transferred to a pin which writes on heat-sensitive paper. As fibrin strands form, the fibrin clot affects movement of the pin. The normal trace shows the rate of initial fibrin formation, the time to formation of a clot (coagulation time), strength of the fibrin clot, clot lysis index or retraction. Typical patterns showing results in fibrinolysis, hypercoagulability, haemophilia and thrombocytopenia are shown in Fig. 24.10.

The platelet function analyser (PFA-100) may also be useful in testing platelet function before and during surgery (p. 277).

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CHAPTER 25

25 Thrombosis and antithrombotic therapy

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Thrombi are solid masses or plugs formed in the circulation from blood constituents. Platelets and fibrin form the basic structure. Their clinical significance results from ischaemia from local vascular obstruction or distant embolization. Thrombi are involved in the pathogenesis of myocardial infarction, cerebrovascular disease, peripheral arterial disease and deep vein occlusion.

Thrombosis, both arterial and venous, is more common as age increases and is frequently associated with risk factors (e.g. surgery or pregnancy). The term thrombophilia is used to describe the inherited or acquired disorders of the haemostatic mechanism that predispose to thrombosis.

Arterial thrombosis

Pathogenesis

Atherosclerosis of the arterial wall, plaque rupture and endothelial injury expose blood to subendothelial collagen and tissue factor. This initiates the formation of a platelet nidus on which platelets adhere and aggregate.

Platelet deposition and thrombus formation are important in the pathogenesis of atherosclerosis. Platelet-derived growth factor (PDGF) stimulates the migration and proliferation of smooth muscle cells and fibroblasts in the arterial intima. Regrowth of endothelium and repair at the site of arterial damage and incorporated thrombus result in thickening of the vessel wall.

As well as blocking arteries locally, emboli of platelets and fibrin may break away from the primary thrombus to occlude distal arteries. Examples are carotid artery thrombi leading to cerebral thrombosis and transient ischaemic attacks (TIAs) and heart valve and chamber thrombi leading to systemic emboli and infarcts (Fig. 25.1).

Clinical risk factors

The risk factors for arterial thrombosis are related to the development of atherosclerosis and are listed in Table 25.1. The identification of patients at risk is largely based on clinical assessment. A number of epidemiological studies have resulted in the construction of coronary artery thrombosis risk profiles based on sex, age, elevated blood pressure, high levels of serum cholesterol, glucose intolerance, cigarette smoking and electrocardiogram (ECG) abnormalities. These profiles have allowed pre-symptomatic assessment of young and apparently fit subjects and are valuable in counselling a change in lifestyle or for recommending medical therapy in individuals at risk. The Northwick Park Heart Study showed

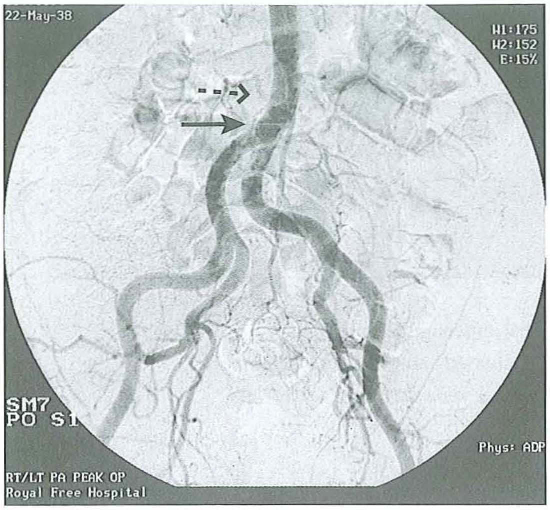


Fig. 25.1 Arteriogram showing saddle embolus at the aortic bifurcation (dotted arrow) and embolus in the left common iliac artery (solid arrow).

Table 25.1 Risk factors in arterial thrombosis (atherosclerosis).

Positive family history
Male sex
Hyperlipidaemia
Hypertension
Diabetes mellitus
Gout
Polycythaemia
Hyperhomocysteinaemia
Cigarette smoking
ECG abnormalities
Elevated factor VII
Elevated fibrinogen
Lupus anticoagulant
Collagen vascular diseases
Behçet's disease

ECG, electrocardiogram.

that elevated plasma levels of factor VII and fibrinogen are the strongest independent predictors of coronary events. Hyperhomocysteinaemia has been recognized more recently as a risk factor for peripheral and coronary arterial disease and stroke.

Venous thrombosis

Pathogenesis and risk factors

Virchow's triad suggests that there are three components that are important in thrombus formation:

Table 25.2 Risk factors for venous thrombosis.

Related to coagulation abnormality
Hereditary haemostatic disorders
Factor V Leiden
Prothrombin G20210A variant
Protein C deficiency
Antithrombin deficiency
Protein S deficiency
Abnormal fibrinogen
Abnormal plasminogen
Hereditary or acquired haemostatic disorders
Raised plasma levels of factor VII, VIII, IX or XI
Raised plasma levels of fibrinogen
Raised plasma levels of homocysteine
Glucosylceramide deficiency
Coagulation factor IX concentrates
Lupus anticoagulant
Oestrogen therapy (oral contraceptive and HRT)
Heparin-induced thrombocytopenia
Pregnancy and puerperium
Surgery, especially abdominal and hip
Major trauma
Malignancy
Myocardial infarct
Thrombocythaemia
Related to stasis
Cardiac failure
Stroke
Prolonged immobility
Pelvic obstruction
Nephrotic syndrome
Dehydration
Hyperviscosity, polycythaemia
Varicose veins
Related to unknown factors
Age
Obesity
Sepsis
Paroxysmal nocturnal haemoglobinuria
Behçet's disease

HRT, hormone replacement therapy.

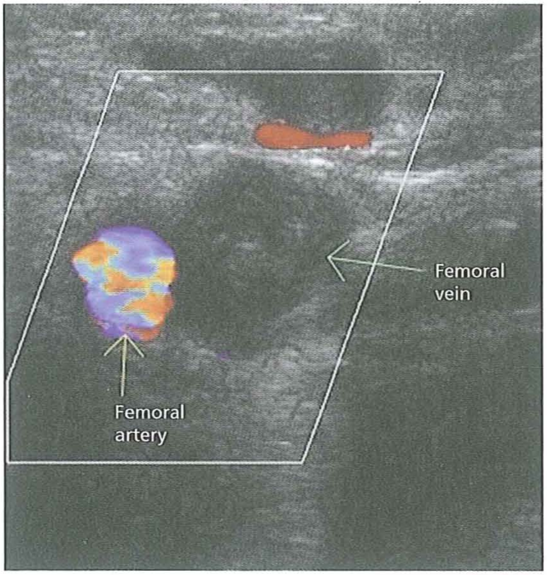
1 Slowing down of blood flow;
2 Hypercoagulability of the blood; and
3 Vessel wall damage.

For venous thrombosis, increased systemic coagulability and stasis are most important, vessel wall damage being less important than in arterial thrombosis, although it may be important in patients with sepsis and indwelling catheters. Stasis allows the completion of blood coagulation at the site of initiation of the thrombus (e.g. behind the valve pockets of the leg veins in immobile patients).

Table 25.2 lists a number of recognized risk factors.

Hereditary disorders of haemostasis

The prevalence of inherited disorders associated with increased risk of thrombosis is at least as high as that of hereditary bleeding disorders. A hereditary 'thrombophilia' should be particularly suspected in young patients who suffer from spontaneous thrombosis, recurrent deep vein thromboses (Fig. 25.2) or



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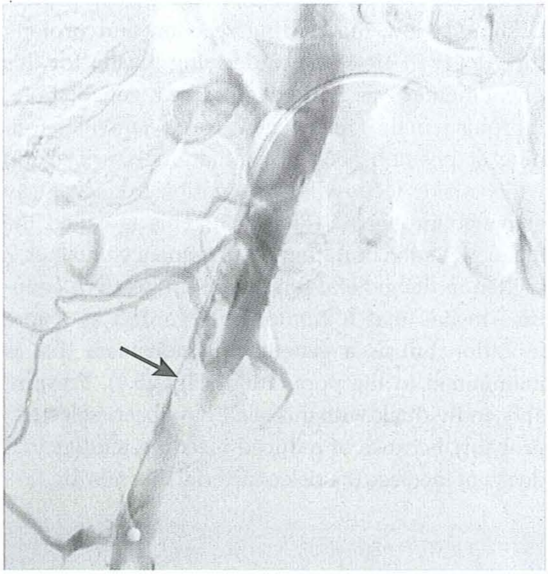

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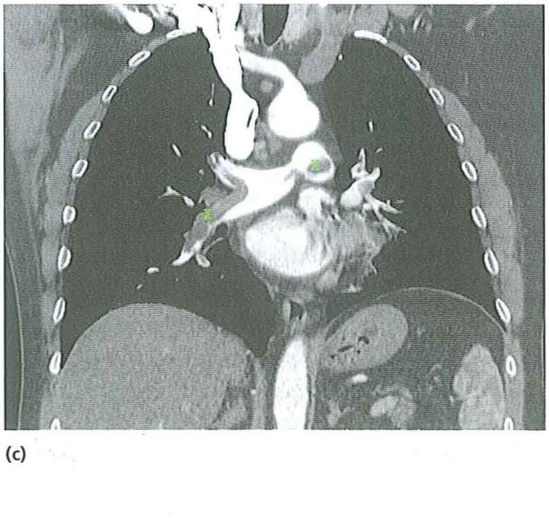

(c)

Fig. 25.2 Diagnostic imaging of deep vein thrombosis (DVT) and pulmonary embolus (PE). **(a)** Colour power Doppler ultrasound of the right femoral vessels with compression shows normal flow in the femoral artery but absent flow in the vein because of thrombus. A normal vein would collapse with compression of the probe. (Courtesy of Dr Tony Young) **(b)** Femoral venogram demonstrating extensive thrombus within the right external iliac vein. (Courtesy of Drs I.S. Francis and A.F. Watkinson) **(c)** Computed tomography (CT) pulmonary angiography: a coronal image shows bilateral filling defects (green crosses) in the central central pulmonary arteries indicating pulmonary emboli. (Courtesy of Dr Tony Young)

an unusual site of thrombosis (e.g. axillary, splanchnic veins, sagittal sinus). Several abnormalities are now well characterized (Table 25.2).

Factor V Leiden gene mutation (activated protein C resistance)

This is the most common inherited cause of an increased risk of venous thrombosis. It occurs in approximately 4% of Caucasian factor V alleles. It was first recognized because of a failure to see prolongation in the activated partial thromboplastin time (APTT) test when activated protein C was added to the plasma of certain patients. Protein C breaks down activated factor V so activated protein C should slow the clotting reaction and prolong the APTT. In 1994, the underlying reason for this phenomenon was recognized to be a genetic polymorphism in the factor V gene (replacement of arginine at position 506 with glutamine—Arg506Gln) which makes factor V less susceptible to cleavage by activated protein C (Fig. 25.3). This is called the factor V Leiden mutation. The frequency of factor V Leiden in the general population in Western countries means that it cannot be regarded as a rare mutation but as a genetic polymorphism that is maintained in the population (Fig. 25.4). Presumably, individuals with this allele have been 'selected', probably because of reduced bleeding tendency. It does not increase the risk of arterial thrombosis.

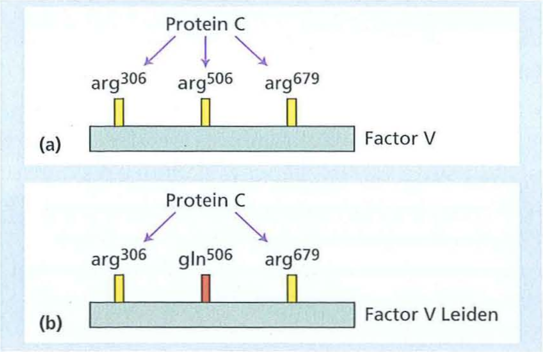

Fig. 25.3 The genetic basis of factor V Leiden. **(a)** Activated protein C inactivates factor Va by proteolytic cleavage at three sites in the Va heavy chain. **(b)** In the factor V Leiden mutation the Arg506Gln polymorphism leads to glutamine at position 506 with less efficient inactivation of factor V and increased risk of thrombosis.

Patients who are heterozygous for factor V Leiden are at an approximately 5–8-fold increased risk of thrombosis compared to the general population. Individuals who are homozygous have a 30–140-fold risk. Following venous thrombosis they have a higher risk of re-thrombosis compared to individuals with deep vein thrombosis (DVT) but normal factor V.

The incidence of factor V Leiden in patients with venous thrombosis is approximately 20–40%. Polymerase chain reaction (PCR) screening for the mutation is relatively simple and the test is widely performed. The absolute risk of thrombosis will depend on many other factors and it is difficult to advise individual patients of their risk. At present it is not recommended to start anticoagulation therapy in individuals with the Leiden mutation, even if homozygous, with no history of thrombosis. A small minority of patients with activated protein C resistance do not have factor V Leiden and have other mutations of factor V.

Antithrombin deficiency

Inheritance is autosomal dominant. There are recurrent venous thromboses usually starting in early adult life. Arterial thrombi occur occasionally. Antithrombin concentrates are available and are used to prevent thrombosis during surgery or childbirth. Many molecular variants of antithrombin have been categorized and are associated with varying degrees of risk of thrombosis.

Protein C deficiency

Inheritance is autosomal dominant with variable penetrance. Protein C levels in heterozygotes are approximately 50% of normal. Characteristically, many patients develop skin necrosis as a result of dermal vessel occlusion when treated with warfarin, thought to be caused by reduction of protein C levels even further in the first day or two of warfarin therapy before reduction in the levels of the vitamin K-dependent clotting factors, especially factor VII. Rarely, infants may be born with homozygous deficiency and characteristically present with severe disseminated intravascular coagulation (DIC) or purpura fulminans in infancy. Protein C concentrates are available.

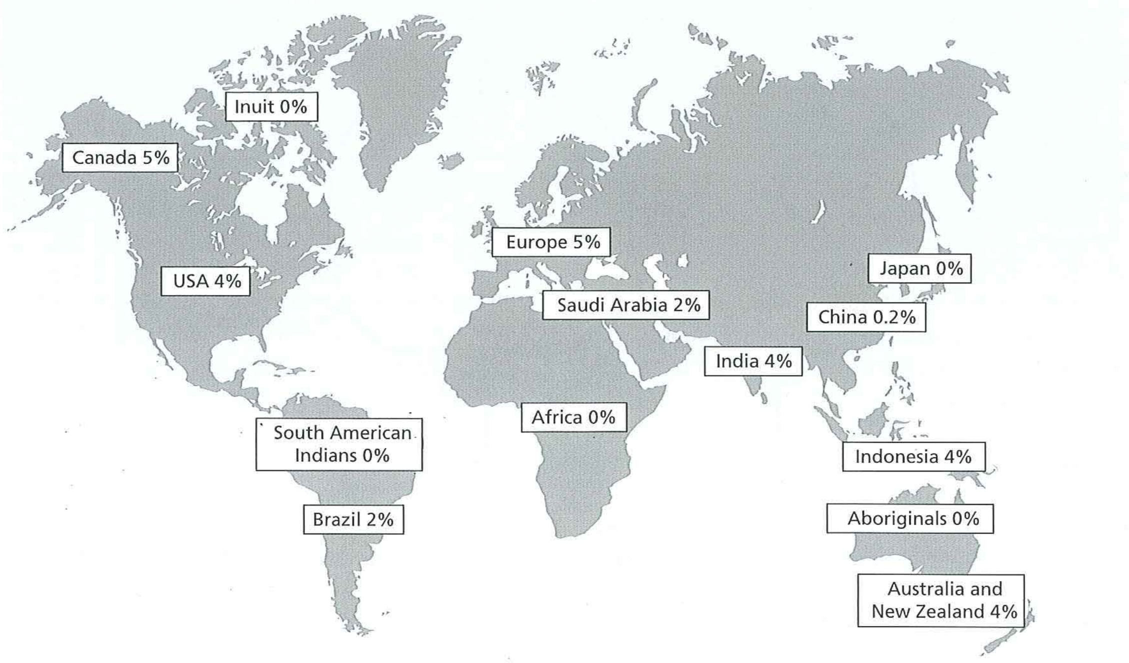

Fig. 25.4 The incidence of carriers of factor V Leiden in different countries.

Protein S deficiency

Protein S deficiency has been found in a number of families with a thrombotic tendency. It is a cofactor for protein C and the clinical features are similar to protein C deficiency, including a tendency to skin necrosis with warfarin therapy. The inheritance is autosomal dominant.

Prothrombin allele G20210A

Prothrombin allele G20210A is a variant (prevalence 2–3% in the population) that leads to increased plasma prothrombin levels and increases thrombotic risk by at least twofold. It is probable that the cause of venous thrombosis with this mutation and with high levels of factors VIII, IX and XI is that sustained generation of thrombin results in prolonged down-regulation of fibrinolysis through activation of thrombin-activated fibrinolysis inhibitor (p. 274).

Hyperhomocyst(e)inaemia

Higher levels of plasma homocysteine may be genetic or acquired and are associated with increased risk for both venous and arterial thrombosis. However, recent large trials show no evidence that lowering the levels reduces these risks.

Homocysteine is derived from dietary methionine and is removed by either remethylation to methionine or conversion to cysteine via a *trans*-sulphuration pathway (Fig. 25.5). Classic homocystinuria is a rare autosomal recessive disorder caused by deficiency of cystathione β-synthase, the enzyme responsible for *trans*-sulphuration. Vascular disease and thrombosis are major features of the disease. Heterozygous cystathione β-synthase deficiency is present in approximately 0.5% of the population and leads to a moderate increase in homocysteine. Methylene tetrahydrofolate reductase (MTHR) is involved in the remethylation pathway and a common thermolabile variant of the enzyme may be responsible for mild homocysteinaemia (above 15 μmol/L) although this may only be seen in the presence of folate deficiency (p. 46). Acquired risk factors for hyperhomocysteinaemia include deficiencies of folate, vitamin B_{12} or vitamin B_6, drugs (e.g. ciclosporin), renal damage

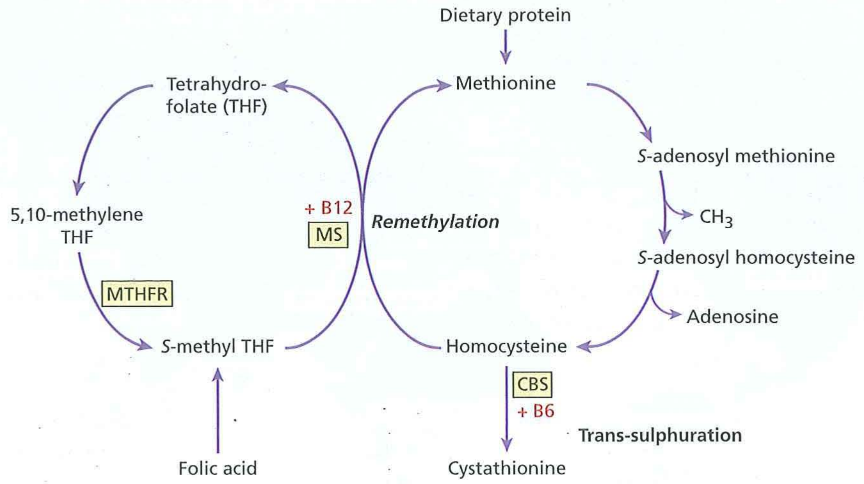

Fig. 25.5 The metabolism of homocysteine. Homocysteine is derived from dietary methionine and is metabolized either by the *trans*-sulphuration or remethylation pathways. *Trans*-sulphuration proceeds using the cystathionine β-synthase (CBS) enzyme with vitamin B_6 as a cofactor. Remethylation involves the action of methionine synthase (MS) on 5-methyl-THF with vitamin B_{12} as a cofactor. In addition, methylene-tetrahydrofolate reductase (MTHFR) is involved in this cycle.

and smoking. The levels also increase with age and are higher in men and post-menopausal females.

Defects of fibrinogen

Defects of fibrinogen are usually clinically silent or cause excess bleeding. Thrombosis is a rare association.

Hereditary or acquired disorders of haemostasis

High factor VIII or fibrinogen levels are also associated arterial thrombosis.

The combination of multiple risk factors is associated with increased risk of thrombosis. If these are persistent they may represent a reason for extended anticoagulation.

Acquired risk factors

These may cause thrombosis in patients without another identifiable abnormality but are most likely to do so if an inherited predisposing abnormality (e.g. factor V Leiden) is also present.

Postoperative venous thrombosis

This is more likely to occur in the elderly, obese, those with a previous or family history of venous thrombosis, and in those in whom major abdominal or hip operations are performed.

Venous stasis and immobility

These factors are probably responsible for the high incidence of postoperative venous thrombosis and for venous thrombosis associated with congestive cardiac failure, myocardial infarction and varicose veins. In atrial fibrillation, thrombin generation from accumulation of activated clotting factors leads to a high risk of systemic embolization. The use of muscle relaxants during anaesthesia may also contribute to venous stasis. Venous thrombosis also has a higher frequency after prolonged aeroplane journeys.

Malignancy

Patients with carcinoma of the ovary, brain and pancreas have a particularly increased risk of venous thrombosis but there is an increased risk with all cancers. The tumours produce tissue factor and a pro-coagulant that directly activates factor X. Mucin-secreting adenocarcinomas may be associated with DIC.

Inflammation

This up-regulates procoagulant factors, down-regulates anticoagulant pathways, particularly

protein C. Thrombosis is particularly likely in inflammatory bowel disease, Behçet's disease, systemic tuberculosis, systemic lupus erythematosus (SLE) and diabetes.

Blood disorders

Increased viscosity, thrombocytosis, altered platelet membrane receptors and responses are possible factors for the high incidence of thrombosis in patients with polycythaemia vera and essential thrombocythaemia. There is a high incidence of venous thrombosis, including thrombi in large veins (e.g. the hepatic vein) in patients with paroxysmal nocturnal haemoglobinuria. An increased tendency to venous thrombosis has been observed in patients with sickle cell disease and patients with post splenectomy thrombocytosis.

Oestrogen therapy

Oestrogen therapy, particularly high-dose therapy, is associated with increased plasma levels of factors II, VII, VIII, IX and X and depressed levels of antithrombin and tissue plasminogen activator in the vessel wall. There is a high incidence of postoperative venous thrombosis in women on high-dose oestrogen therapy and full-dose oestrogen-containing oral contraceptives. The risk is much less with low-dose oestrogen contraceptive preparations. Hormone replacement therapy also increases the risk of thrombosis, largely obviated by the use of low oestrogen preparations.

The antiphospholipid syndrome

This may be defined as the occurrence of thrombosis or recurrent miscarriage in association with laboratory evidence of persistent antiphospholipid antibody. One antiphospholipid antibody is the 'lupus anticoagulant' (LA) which was initially detected in patients with SLE, and is identified by a prolonged plasma APTT which does not correct with a 50 : 50 mixture of normal plasma. A second test dependent on limiting quantities of phospholipid (e.g. the dilute Russell's viper venom test) is also used in diagnosis. The antibody represents part of the spectrum of antiphospholipid antibody syndrome (APS) and whereas lupus anticoagulants are reactive in the fluid phase, the other antiphospholipid antibodies (APAs), such as anticardiolipin antibodies (ACA) and antibodies to β_2-glycoprotein (β_2-GPI), are identified by solid phase immunoassay. Both solid phase assays and coagulation tests for LA should be used in the diagnosis of APS.

Table 25.3 Clinical associations of lupus anticoagulant and anticardiolipin antibodies.

Venous thrombosis deep venous thrombosis/pulmonary embolism renal, hepatic, retinal veins
Arterial thrombosis
Recurrent fetal loss
Thrombocytopenia
Livedo reticularis

NB. Recurrent fetal loss may also occur in other types of thrombophilia.

As well as patients with SLE, APS is also found in other autoimmune disorders particularly of connective tissues, lymphoproliferative diseases, post-viral infections, with certain drugs including phenothiazines and as an 'idiopathic' phenomenon in otherwise healthy subjects. Paradoxically, in view of its name, it is associated with venous and arterial thrombosis. The arterial thrombosis may cause peripheral limb ischaemia, stroke or myocardial infarct. As with other causes of thrombophilia, recurrent abortion caused by placental infarction is also associated (Table 25.3). Thrombocytopenia may be present and livedo reticularis is a frequent dermal manifestation. Treatment is with anticoagulation where indicated. It is usual to maintain an international normalized ratio (INR) of between 2.0 and 3.0 with warfarin but higher levels may be needed if previous arterial or major DVT has occurred or recurrence of thrombosis occurs on warfarin therapy. Low-dose heparin and aspirin are useful in the management of recurrent miscarriage.

Collagen vascular diseases and Behçet's syndrome are also associated with arterial and venous thrombosis, whether or not the lupus anticoagulant is present (p. 300).

Factor IX concentrates

Venous thrombosis may complicate the use of factor IX concentrates which contain trace amounts of activated coagulation factors. Patients with liver disease who are unable to clear these activated factors are especially at risk.

Glucosylceramide deficiency

Reduced plasma levels of the glycolipid glucosylceramide are a potential risk factor for venous thrombosis, particularly in young male patients. The glycolipid modulates the protein C pathway.

Investigation of thrombophilia

Many of the conditions associated with an increased thrombotic risk are obvious following clinical examination. A full assessment is indicated, particularly in patients who have recurrent or spontaneous DVT or pulmonary emboli, in patients who have thrombosis at a young age and in those patients with a familial tendency to thrombosis or thrombosis at an unusual site. It is also needed in women with recurrent fetal loss. With the increasing recognition of systemic causes of thrombophilia, the indications for thrombophilia screening are widening. The following laboratory tests are used in diagnosis.

Screening tests

1 Blood count and erythrocyte sedimentation rate (ESR)—to detect elevation in haematocrit, white cell count, platelet count, fibrinogen and globulins.

2 Blood film examination—may provide evidence of myeloproliferative disorder; leucoerythroblastic features may indicate malignant disease.

3 Prothrombin time (PT) and APTT—a shortened APPT is often seen in thrombotic states and may indicate the presence of activated clotting factors. A prolonged APTT test, not corrected by the addition of normal plasma, suggests a 'lupus anticoagulant' or an acquired inhibitor to a coagulation factor.

4 Anticardiolipin and anti-β_2-GPI antibodies.

5 Thrombin time and reptilase time—prolongation suggests an abnormal fibrinogen.

6 Fibrinogen assay.

7 Activated protein C (APC) resistance test and DNA analysis for factor V Leiden.

8 Antithrombin—immunological and functional assays.

9 Protein C and protein S—immunological and functional assays.

10 Prothrombin gene analysis for the G20210A variant.

11 Plasma homocysteine estimation.

In many patients even full investigation yields no abnormalities and treatment with oral anticoagulants may remain empirical.

12 Test for CD59 and CD55 expression (paroxysmal nocturnal haemoglobinuria) in red cells if paroxysmal nocturnal haemoglobinuria is suspected.

Diagnosis of venous thrombosis

Deep vein thrombosis

Clinical suspicion DVT is suspected in those with previous DVT, cancer or confined to bed. In the leg, unilateral thigh or calf swelling or tenderness, pitting oedema and the presence of collateral superficial non-varicose veins are important signs.

Serial compression ultrasound This is the most reliable and practical method for patients with first suspicion of DVT in the legs and other sites (Fig. 25.2a). It can be combined with spectral, colour (Fig. 25.2) or power Doppler (duplex) scanning which improves accuracy by focusing on individual veins. It does not distinguish between acute and chronic thrombi. Persisting venous obstruction detected by ultrasonography at the completion of warfarin therapy is associated with an increased risk of recurrent thrombosis.

Contrast venography This most sensitive procedure is reserved for patients with highly suggestive clinical findings but negative ultrasonography. Iodinated contrast medium is injected into a vein peripheral to the suspected DVT. This permits direct demonstration by X-ray of the site, size and extent of the thrombus (Fig. 25.2b). However, it is a painful invasive technique, with a risk of contrast reaction and procedure-induced DVT.

Plasma D-dimer concentration The concentration of these fibrin breakdown products is raised when there is a fresh venous thrombosis. It is a useful assay when recurrent thrombosis is suspected and also may be combined with the tests above for diagnosis of a first event. A negative result is useful in emergency departments for excluding DVT in patients with equivocal clinical features. D-dimer elevation in cancer, inflammation after surgery or trauma limits its usefulness.

Magnetic resonance imaging (MRI) This may also be used but is expensive. Impedance plethysmography is less sensitive and accurate and is falling out of use.

Pulmonary embolus

Clinical suspicion This is particularly suspected in patients with chest symptoms, especially if there are signs of or previous history of DVT, immobilization for more than 2 days or recent (<4 weeks) surgery, haemoptysis or cancer.

Chest X-ray This is often normal but may show evidence of pulmonary infarction or pleural effusion.

Ventilation perfusion (VQ) scintigraphy This detects areas of the lung being ventilated but not perfused.

Computed tomography (CT) pulmonary angiography Fine slices of the lung are scanned by spiral CT so that filling defects in the pulmonary arteries are visualized (Fig. 25.2c).

Magnetic resonance pulmonary angiography Gadolinium-enhanced MRI is a relatively new, expensive but accurate technique.

Pulmonary angiography This is the traditional reference method but is invasive with complications, albeit uncommon, such as arrhythmia or contrast reaction.

Electrocardiogram This is performed to determine whether there is right heart 'strain' which occurs only in relatively severe cases.

Anticoagulant drugs

Anticoagulant drugs are used widely in the treatment of venous thromboembolic disease. Their value in the treatment of arterial thrombosis is less well established.

Heparin

This acidic, unfractionated mucopolysaccharide of average molecular weight (MW) 15 000–18 000 is an inhibitor of blood coagulation because of its action in potentiating the activity of antithrombin (see below). As it is not absorbed from the gastrointestinal tract it must be given by injection. It is inactivated by the liver and excreted in the urine. The effective biological half-life is approximately 1 h (Table 25.4).

Table 25.4 Comparison of unfractionated heparin with low molecular weight heparin.

	Unfractionated heparin	Low molecular weight heparin
Mean molecular weight in kilodaltons (range)	15 (4–30)	4.5 (2–10)
Anti-Xa : anti-IIa	1 : 1	2 : 1 to 4 : 1
Inhibits platelet function	Yes	No
Bioavailability	50%	100%
Half-life		
intravenous	1 h	2 h
subcutaneous	2 h	4 h
Elimination	Renal and hepatic	Renal
Monitoring	APTT	Xa assay (usually not needed)
Frequency of heparin-induced thrombocytopenia	High	Low
Osteoporosis	Yes	Less frequent

APTT, activated partial thromboplastin time.

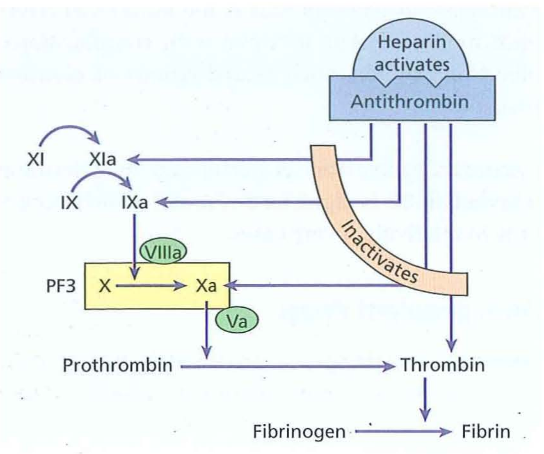

Fig. 25.6 The action of heparin. This activates antithrombin which then forms complexes with activated serine protease coagulation factors (thrombin, Xa, IXa and XIa) and so inactivates them.

Mode of action

Heparin dramatically potentiates the formation of complexes between antithrombin and activated serine protease coagulation factors, thrombin (IIa) and factors IXa, Xa and XIa (Fig. 25.6). This complex formation inactivates these factors irreversibly. In addition, heparin impairs platelet function.

Low molecular weight heparin (LMWH) preparations (MW 2000–10 000) are produced by enzymatic or chemical depolymerization of unfractionated heparin. They have a greater ability to inhibit factor Xa than to inhibit thrombin and interact less with platelets compared with standard heparin, and so may have a lesser tendency to cause bleeding. They also have greater bioavailability and a more prolonged half-life in plasma, making once-daily administration in prophylaxis or treatment feasible (Table 25.4).

Indications

Heparin is routinely used in DVT, pulmonary embolism and unstable angina pectoris. It is also widely used in the prophylaxis of venous thrombosis and is the drug of choice for women requiring anticoagulation in pregnancy because it does not cross the placenta. It is also used during cardiopulmonary bypass surgery, for maintaining the patency of indwelling venous lines and in some case of DIC if the manifestations are predominantly vaso-occlusive. There is some evidence that LMWH (or warfarin) may improve the survival in cancer patients over and above the protection from venous thrombosis but the exact mechanisms, types of cancer improved and optimum treatment regimes remain to be elucidated.

Administration and laboratory control

Standard heparin

Continuous intravenous infusion This provides the smoothest control of heparin therapy and is the treatment of choice where rapid reversal of anticoagulation by protamine sulphate may be required (e.g. in surgical patients or late pregnancy). It is still preferred by many for treatment of acute pulmonary embolus. In an adult, dosage of 30 000–40 000 units over 24 h (1000–2000 units/h with a loading dose of 5000 units) is usually satisfactory. Therapy is monitored by maintaining the APTT at 2–3 times the upper limit of the normal value. It is usual to start warfarin therapy within 2 days of starting heparin therapy and to discontinue heparin when the INR has been above 2.0 on two successive days. For acute coronary syndromes, both unfractionated heparin and LMWH are of benefit when used with aspirin in the prevention of mural thrombosis, systemic embolization and venous thrombosis.

Subcutaneous heparin Intermittent subcutaneous injections are preferred when heparin is given as prophylaxis against venous thrombosis (e.g. for surgical procedures). The usual dosage is 5000 units 12-hourly preoperatively followed by this dosage 8–12-hourly for 7 days or until the patient is mobile. LMWH (see below) is usually perferred as it can be given once daily.

Low molecular weight heparin

LMWH is given by subcutaneous injection and, as it has a longer half-life than standard heparin, it can be given once a day in prophylaxis, or once or twice a day in treatment (Table 25.4). Compared with unfractionated heparin, LMWH has a more

predictable dose–response which avoids the need for routine monitoring. There is less risk of thrombocytopenia or osteoporosis. It is the treatment of choice for preventing or treating DVT. It is used increasingly for treatment of pulmonary embolus and unstable angina. Although routine monitoring is not required, measurement of anti-Xa peak levels 4 h after injection allows dose adjustment in selected patients (e.g. in pregnancy, renal failure, gross obesity and in children). Typical treatment regimens are 200 anti-Xa units/kg once daily or 100 anti-Xa units/kg twice daily). LMWH is preferred to unfractionated heparin for therapy of DVT including pulmonary embolism. Many patients with uncomplicated DVTs may now be managed at home with regular LMWH injections once or twice daily according to the preparation. LMWH is the preferred treatment for the prevention of DVT in both medical and surgical patients. It is also the preferred anticoagulant in pregnancy because it does not cross the placenta. Typical once daily subcutaneous dosage in prophylaxis is 2000–2500 units (moderate risk patients) or 4000–5000 units (high-risk patients).

Complications

Unfractionated heparin is associated with a number of side-effects. The risk of these complications appears to be reduced by approximately 50% by the use of LMWH.

Bleeding during heparin therapy

Bleeding may be because of excessive prolonged anticoagulation or to an antiplatelet functional effect of heparin. Intravenous heparin has a half-life of less than 1 h and it is usually only necessary to stop the infusion. Protamine is able to inactivate heparin immediately and for severe bleeding a dose of 1 mg/100 units heparin provides effective neutralization. However, protamine itself may act as an anticoagulant when in excess.

Heparin-induced thombocytopenia

A mild lowering of the platelet count may occur in the first 24 h as a result of platelet clumping. This is of no clinical consequence (heparin-induced thombocytopenia, HIT, type 1). The important HIT, type 2, may occur in up to 5% of patients who are treated with unfractionated heparin and paradoxically presents with thrombosis. It results from the binding of heparin to platelets followed by the generation of an immunoglobulin G (IgG) antibody to the heparin–platelet factor 4 (PF4) complex, which leads to platelet activation (Fig. 25.7). Typically, it presents as a fall of >50% in the platelet count 5 or more days after starting heparin treatment or earlier if heparin has been given previously. Diagnosis is difficult but assays have recently been developed to allow the detection of antibodies to immobilized heparin–PF4 complex. Heparin therapy must be discontinued. Thrombin inhibitors such as hirudin or lepirudin or argatroban appear promising as alternatives and the heparinoid danaparoid may also be used. LMWH is less likely than unfractionated heparin to cause HIT but there is cross-reactivity of the antibody. Warfarin therapy in some cases causes skin necrosis and should be delayed until alternative anticoagulation has been achieved.

Osteoporosis

This occurs with long-term (>2 months) heparin therapy, especially in pregnancy. The drug complexes minerals from the bones but the exact pathogenesis is unknown.

Oral anticoagulants

These are derivatives of coumarin or indandione. Warfarin, a coumarin, is most widely used. The drugs are vitamin K antagonists (p. 297) and so treatment results in decreased biological activity of the vitamin K-dependent factors II, VII, IX and X. Oral anticoagulants block the post-ribosomal γ-carboxylation of glutamic acid residues of these proteins (Fig. 24.7). After warfarin is given, factor VII levels fall considerably within 24 h but prothrombin has a longer plasma half-life and only falls to 50% of normal at 3 days; the patient is fully anticoagulated only after this period.

Principles of oral anticoagulation

A typical starting regimen for warfarin would be 10 mg on day 1, 5 mg on day 2 and then 5 mg on the third day. After this the dosage should be adjusted according to the PT. The usual maintenance dosage

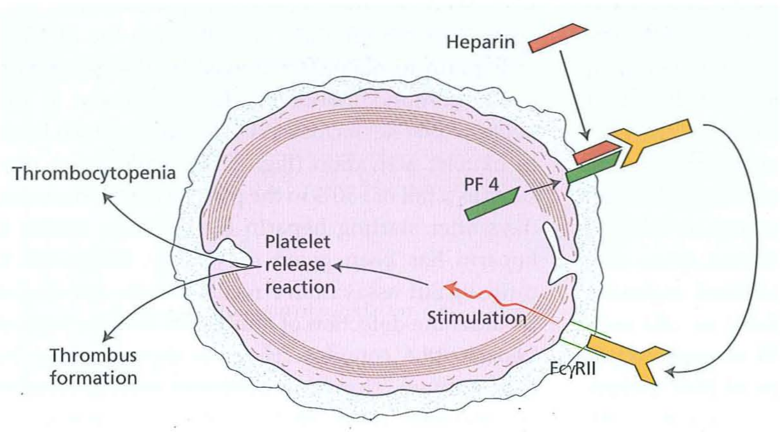

Fig. 25.7 Mechanism of heparin-induced thrombocytopenia (HIT). Platelet factor 4 (PF4) is released from α granules and forms a complex on the platelet surface with heparin. Immunoglobulin G antibodies (usually IgG_2) develop against this complex and once bound can activate the platelet through the platelet immunoglobulin receptor Fc-γRII. This leads to platelet stimulation, further release of PF4 and the platelet release reaction with consequent thrombocytopenia and thrombus development.

Table 25.5 Oral anticoagulant control tests. Target levels recommended by the British Society for Haematology (2000).

Target INR	Clinical state
2.5 (2.0–3.0)	Treatment of DVT, pulmonary embolism, atrial fibrillation, recurrent DVT off warfarin; symptomatic inherited thrombophilia, cardiomyopathy, mural thrombus, cardioversion
3.5 (3.0–4.0)	Recurrent DVT while on warfarin, mechanical prosthetic heart valves, antiphospholipid syndrome (some cases)

DVT, deep vein thrombosis; INR, international normalized ratio.

of warfarin is 3–9 mg/day but individual responses vary greatly. Lower loading dosage is recommended for the elderly or those with liver disease.

The indications and recommended ranges for INR with warfarin treatment are summarized in Table 25.5. The effect of oral anticoagulants is monitored by the PT. The INR is used and is based on the ratio of the patient's PT to a mean normal PT with correction for the 'sensitivity' of the thromboplastin used. This is calibrated against a primary World Health Organization (WHO) standard thromboplastin.

Warfarin crosses the placenta and is teratogenic. Heparin is preferred for pregnant patients because it does not cross the placenta and its action is short-lived.

It is usual to continue warfarin for 3–6 months for established DVT, pulmonary embolism and following xenograft heart valves. Long-term therapy is given for recurrent venous thrombosis, for embolic complications of rheumatic heart disease or atrial fibrillation, and with prosthetic valves and arterial grafts. It is also given long term in patients with a severe cause of thrombophilia (e.g. the lupus anticoagulant and a history of thrombosis).

Drug interactions

Approximately 97% of warfarin in the circulation is bound to albumin and only a small fraction of warfarin is free and can enter the liver parenchymal cells; it is this free fraction that is active. In the liver cells, warfarin is degraded in microsomes to an inactive water-soluble metabolite which is conjugated and excreted in the bile and partially reabsorbed to be also excreted in urine. Drugs that affect the albumin binding or excretion of warfarin (or of other oral anticoagulants) or those that decrease the absorption of vitamin K will interfere with the control of therapy (Table 25.6).

Table 25.6 Drugs and other factors that interfere with the control of anticoagulant therapy.

Potentiation of oral anticoagulants	Inhibition of oral anticoagulants
Drugs that increase the effect of coumarins	**Drugs that depress the action of coumarins**
Reduced coumarin binding to serum albumin	*Acceleration of hepatic microsomal degradation of coumarin*
Sulfonamides	Barbiturates
Inhibition of hepatic microsomal degradation of coumarin	Rifampicin
Cimetidine	*Enhanced synthesis of clotting factors*
Allopurinol	Oral contraceptives
Tricyclic antidepressants	**Hereditary resistance to oral anticoagulants**
Metronidazole	
Sulfonamides	**Pregnancy**
Alteration of hepatic receptor site for drug	
Thyroxine	
Quinidine	
Decreased synthesis of vitamin K factors	
High doses of salicylates	
Some cephalosporins	
Liver disease	
Decreased synthesis of vitamin K factors	
Decreased absorption of vitamin K	
e.g. malabsorption, antibiotic therapy, laxatives	

NB. Patients are also more likely to bleed if taking antiplatelet agents (e.g. NSAIDs, dipyridamole or aspirin); alcohol in large amounts enhances warfarin action.

Management of warfarin overdose

If the INR is in excess of 4.5 without bleeding, warfarin should be stopped for 1 or 2 days and the dose adjusted according to the INR. The long half-life of warfarin (40 h) delays the full impact of dose changes for 4–5 days. If the INR is very high (e.g. >8) without bleeding, an oral dose of 0.5–2.5 mg vitamin K may be given. Mild bleeding usually only needs an INR assessment, drug withdrawal and subsequent dosage adjustment (Table 25.7). More serious bleeding may need cessation of therapy, vitamin K therapy or the infusion of fresh frozen plasma or prothrombin concentrates. Vitamin K is the specific antidote; an oral or intravenous dose of 2.5 mg is usually effective. Higher doses result in resistance to further warfarin therapy for 2–3 weeks.

Management of surgery

For minor surgery (e.g. dental extraction) anticoagulation can be maintained and mouth rinses with tranexamine acid given. For major surgery, warfarin is stopped to get an INR <1.5 and LMWH given when the INR falls to <2.0 (except on the day or surgery) and continued until the INR is >2.0 after restarting warfarin.

New anticoagulants

Traditional anticoagulant therapy has disadvantages. LMWH has to be given subcutaneously and warfarin requires frequent monitoring and dose adjustment and there is interaction with drugs and food.

Factor Xa inhibitors: Fondaparinux, a synthetic analogue of the antithrombin-binding pentasaccharide of heparin, is an indirect irreversible factor Xa inhibitor. It is given subcutaneously, has a plasma half-life of 17 h and does not require laboratory monitoring. Extensive trials have demonstrated that

Table 25.7 Recommendations on the management of bleeding and excessive anticoagulation by the British Committee for Standards in Haematology (third edition 1998; 2005 update).

INR 3.0–6.0 (target INR 2.5)	Reduce warfarin dose or stop
INR 4.0–6.0 (target INR 3.5)	Restart warfarin when INR <5.0
INR 6.0–8.0 No bleeding or minor bleeding	Stop warfarin* Restart when INR <5.0
INR >8.0 No bleeding or minor bleeding	Stop warfarin Restart warfarin when INR <5.0 If other risk factors for bleeding give 0.5–2.5 mg vitamin K orally
Major bleeding	Stop warfarin Give prothrombin complex concentrate 50 units/kg, in preference FFP 15 mL/kg (when available) Give 5 mg vitamin K (i.v. or oral)

FFP, fresh frozen plasma; INR, international normalized ratio; i.v., intravenous.
* 1 mg vitamin K may be given orally to rapidly reduce the INR to the therapeutic range within 24 h in all patients with an INR above the therapeutic range and no bleeding.

it is an effective antithrombotic agent in selected patients for prevention and treatment of both venous and arterial thromboembolic disorders. It also reduces major bleeding and may improve long-term mortality and morbity e.g. in acute coronary syndromes. *Idraparinux*, a chemically modified analogue of fondaparinux has a longer half-life and, as a once weekly injection, it may be more convenient than LMWH in treatment and prophylaxis, and has the potential to replace warfarin in selected patients.

Direct thrombin inhibitors These include recombinant hirudins, bivalirudin, argatroban, melagratan and ximelagratan.

Bivalirudin has been used as an alternative to heparin in patients undergoing percutaneous coronary interventions and is associated with less bleeding and a reduced need for adjunctive treatment with glycoprotein IIb/IIIa antagonists in patients undergoing stenting.

Ximelagratan, a lipophilic pro-drug may be given orally and following absorption is rapidly converted to melagratan. *Melagratan* may be given subcutaneously. Routine anticoagulation monitoring is unnecessary. Approximately 6–10% of patients develop reversible increases in liver enzymes. Ximelagratan has been compared with either LMWH or warfarin in large numbers of clinical trials. It is as effective as conventional therapy in preventing venous thrombous in orthopoedic and general surgery without an increased risk of major haemorrhage. It has had similar success in the treatment of acute venous thrombosis, myocardial infarction and severe recurrent myocardial ischaemia and in the prevention of stroke and systemic arterial embolism in patients with atrial fibrillation.

Before they are licensed, more data on their effectiveness and safety in a more diverse spectrum of patients are necessary. No specific antidote is available for either the pentasaccharides or ximelagatran.

Graduated compression stockings

These are used postoperatively and during long aeroplane flights to reduce the risk of DVT. After a DVT, they reduce the risk of post-thrombotic syndrome.

Inferior vena cava filter

This can provide short-term protection against pulmonary embolism when a DVT in the legs is diagnosed but anticoagulation is contraindicated (e.g. ongoing or very recent intracranial or gastrointestinal bleeding or where there is recurrent pulmonary embolism despite adequate anticoagulation).

Table 25.8 Fibrinol

Streptokinase (SK)
Tissue plasminogen
Single-chain urokin (SCU-PA)
Acylated plasminog (APSAC)

Fibrinolytic ag

A number of fibrir thrombi (Table 25 ally for patients v major pulmonary bosis, and locally arterial occlusion.

Administration simplified with The therapy is m symptoms begin Streptokinase is g units followed b units/h for 12–48 streptokinase is g units over 60 min. the value of additic

The use of lab control of short-te considered unnec complications ex agents (Table 25.9

Table 25.9 Contrain

Absolute contraindic
Active gastrointestir
Aortic dissection
Head injury or cereb in the past 2 montl
Neurosurgery in the
Intracranial aneurys
Proliferative diabeti

Table 25.10 Antiplatelet therapy in patients with an acute coronary syndrome and in those undergoing percutaneous coronary intervention (PCI). (After Lange R.A. and Hillis L.D. (2004) Antiplatelet therapy for ischemic heart disease. *N Engl J Med* **350**,277–80)

Drug	Target/patient group	Duration
Acute coronary syndrome		
Aspirin	All	Life long
Clopidogrel	All	9–12 months
Glycoprotein IIb/IIIa inhibitors		
Abciximab	None	–
Eptifibatide	High risk	48–72 h
Tirofiban	High risk	48–72 h
Patients undergoing PCI		
Aspirin	All	Life long
Clopidogrel	All	9–12 months
Abciximab	High risk	12 h after PCI
Eptifibatide	High risk	18–24 h after PCI
Tirofiban	None	–

monophosphate (cAMP) levels in circulating platelets which decreases their sensitivity to activating stimuli. Dipyridamole has been shown to reduce thromboembolic complications in patients with prosthetic heart valves and to improve the results in coronary bypass operations.

Sulfinpyrazone This drug is a competitive inhibitor of cyclo-oxygenase. It has been effective in reducing the frequency of blockage in arteriovenous shunts in chronic dialysis patients.

Ticlopidine This is an antiplatelet drug that was used following coronary angioplasty. Side-effects include neutropenia or thrombocytopenia. It has been replaced by clopidogrel except in patients intolerant of clopidogrel.

Clopidogrel This adenosine diphospate (ADP) receptor antagonist is an antiplatelet agent in use for reduction of ischaemic events in patients with

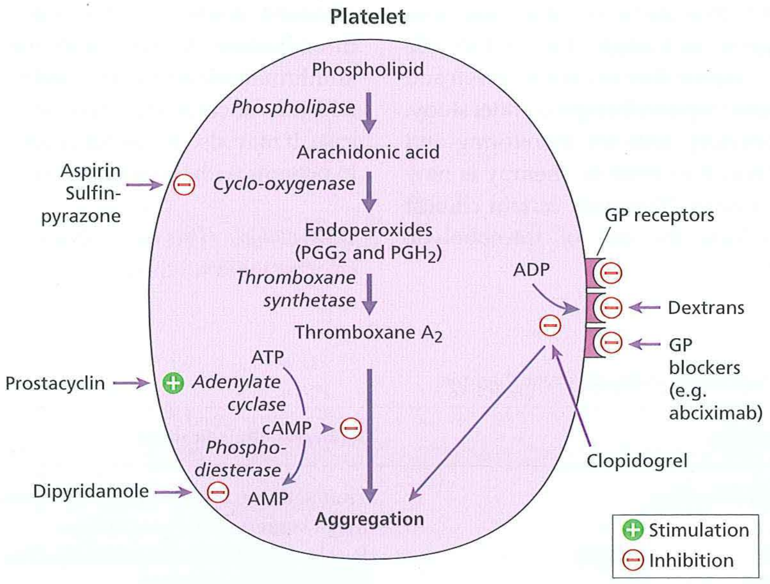

Fig. 25.8 Sites of action of antiplatelet drugs. Aspirin acetylates the enzyme cyclo-oxygenase irreversibly. Sulfinpyrazone inhibits cyclo-oxygenase reversibly. Dipyridamole inhibits phosphodiesterase, increases cyclic adenosine monophosphate (cAMP) levels and inhibits aggregation. Inhibition of adenosine uptake by red cells allows adenosine accumulation in plasma which stimulates platelet adenylate cyclase. Prostacyclin (epoprostenol) stimulates adenylate cyclase. The lipid-soluble β-blockers inhibit phospholipase. Calcium-channel antagonists block the influx of free calcium ions across the platelet membrane. Dextrans coat the surface interfering with adhesion and aggregation. GP, glycoprotein.

ischaemic stroke, myocardial infarction or peripheral vascular disease. It is used after coronary artery stenting or angioplasty and in patients requiring long-term antiplatelet therapy who are intolerant or allergic to aspirin.

Glycoprotein IIb/IIIa inhibitors: abciximab, eptifibatide, tirofiban These drugs are monoclonal antibodies that inhibit the platelet GPIIb/IIIa receptor. They are used in conjunction with heparin and aspirin for the prevention of ischaemic complications in high-risk patients undergoing percutaneous transluminal coronary angioplasty. They can be used once only.

Specific antithrombotic agents Intravenous prostacyclin has been used in clinical trials in patients with peripheral vascular disease and thrombotic thrombocytopenic purpura. It has also reduced arteriovenous shunt blockage in haemodialysis patients.

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CHAPTER 26

26 Haematological changes in systemic disease

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Anaemia of chronic disorders

Many of the anaemias seen in clinical practice occur in patients with systemic disorders and are the result of a number of contributing factors. The anaemia of chronic disorders (also discussed on p. 39) is of central importance and occurs in patients with a variety of chronic inflammatory and malignant diseases (Table 26.1). Usually, both the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are raised. It may be complicated by additional features which may be because of disease affecting particularly one or other system. The characteristic features are described in Chapter 3.

The pathogenesis of this anaemia appears to be related to the decreased release of iron from macrophages to plasma and so to erythroblasts caused by hepcidin, reduced red cell lifespan and an inadequate erythropoietin response to anaemia. The plasma levels of various cytokines, especially interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF) are raised and reduce erythropoietin secretion. The anaemia is corrected by the successful treatment of the underlying disease. It does not respond to iron therapy despite the low serum iron. Responses to recombinant erythropoietin therapy may be obtained (e.g. in rheumatoid arthritis or cancer). In many conditions the anaemia is complicated by anaemia from other causes (e.g. iron or folate deficiency, renal failure, bone marrow infiltration, hypersplenism or endocrine abnormality).

Table 26.1 Causes of anaemia of chronic disorders.

Chronic inflammatory diseases

Infectious (e.g. pulmonary abscess, tuberculosis, osteomyelitis, pneumonia, bacterial endocarditis)

Non-infectious (e.g. rheumatoid arthritis, systemic lupus erythematosus and other connective tissue diseases, sarcoid, Crohn's disease, cirrhosis)

Malignant disease

(e.g. carcinoma, lymphoma, sarcoma, myeloma)

Malignant diseases (other than primary bone marrow diseases)

Anaemia

Contributing factors include anaemia of chronic disorders, blood loss and iron deficiency, marrow infiltration (Fig. 26.1) often associated with a leucoerythroblastic blood film (nucleated red cells and granuloctye precursors in the blood film), folate deficiency, haemolysis and marrow suppression from radiotherapy or chemotherapy (Table 26.2). Other causes of a leucoerythroblastic anaemia include

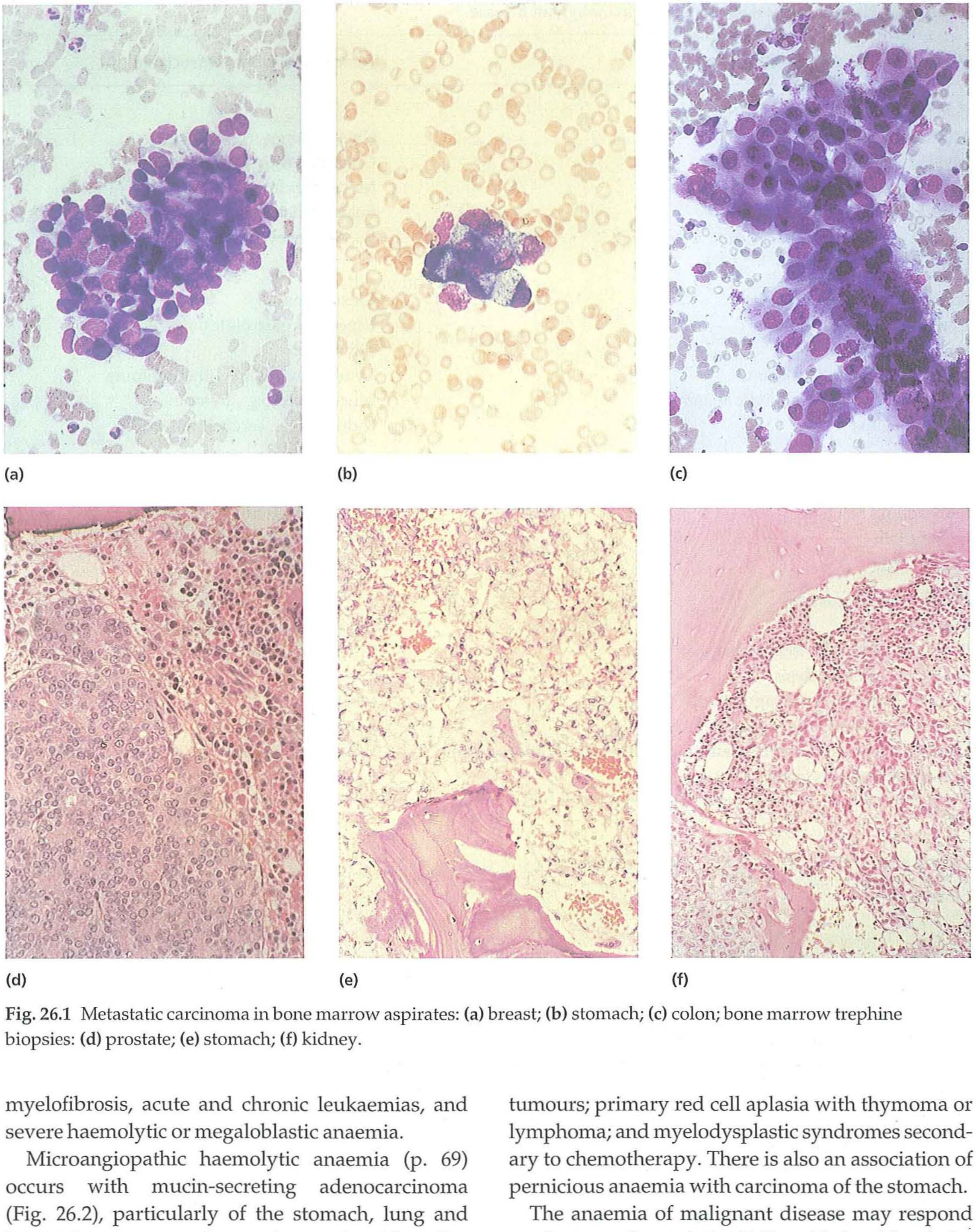


Fig. 26.1 Metastatic carcinoma in bone marrow aspirates: **(a)** breast; **(b)** stomach; **(c)** colon; bone marrow trephine biopsies: **(d)** prostate; **(e)** stomach; **(f)** kidney.

myelofibrosis, acute and chronic leukaemias, and severe haemolytic or megaloblastic anaemia.

Microangiopathic haemolytic anaemia (p. 69) occurs with mucin-secreting adenocarcinoma (Fig. 26.2), particularly of the stomach, lung and breast. Less common forms of anaemia with malignant disease include autoimmune haemolytic anaemia with malignant lymphoma and rarely with other tumours; primary red cell aplasia with thymoma or lymphoma; and myelodysplastic syndromes secondary to chemotherapy. There is also an association of pernicious anaemia with carcinoma of the stomach.

The anaemia of malignant disease may respond partly to erythropoietin. Folic acid should only be given if there is definite megaloblastic anaemia caused by the deficiency; it might 'feed' the tumour.

Table 26.2 Haematological abnormalities in malignant disease.

Haematological abnormality	Tumour or treatment associated
Pancytopenia	
Marrow hypoplasia	Chemotherapy, radiotherapy
Myelodysplasia	Chemotherapy, radiotherapy
Leucoerythroblastic	Metastases in marrow
Megaloblastic	Folate deficiency
	B_{12} deficiency (carcinoma of stomach)
Red cells	
Anaemia of chronic disorders	Most forms
Iron deficiency anaemia	Especially gastrointestinal, uterine
Pure red cell aplasia	Thymoma
Immune haemolytic anaemia	Lymphoma, ovary, other tumours
Microangiopathic haemolytic anaemia	Mucin-secreting carcinoma
Polycythaemia	Kidney, liver, cerebellum, uterus
White cells	
Neutrophil leucocytosis	Most forms
Leukaemoid reaction	Disseminated tumours, those with necrosis
Eosinophilia	Hodgkin's disease, others
Monocytosis	Various tumours
Platelets and coagulation	
Thrombocytosis	Gastrointestinal tumours with bleeding, others
Disseminated intravascular coagulation	Mucin-secreting carcinoma, prostate
Activation of fibrinolysis	Prostate
Acquired inhibitors of coagulation	Most forms
Paraprotein interfering with platelet function	Lymphomas, myeloma
Tumour cell procoagulants—tissue factor and cancer procoagulant (density activates factor X)	Especially ovarian, pancreas, brain, colon

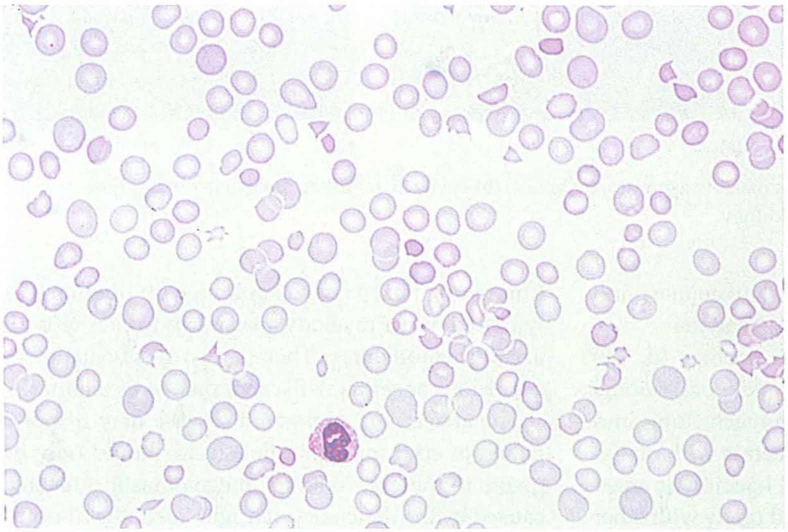


Fig. 26.2 Peripheral blood film in metastatic mucin-secreting adenocarcinoma of the stomach showing red cell polychromasia and fragmentation and thrombocytopenia. The patient had disseminated intravascular coagulation.

Polycythaemia

Secondary polycythaemia is occasionally associated with renal, hepatic, cerebellar and uterine tumours (p. 236).

White cell changes

Leukaemoid reactions (p. 102) may occur with tumours showing widespread necrosis and inflammation. Hodgkin's disease is associated with a variety of white cell abnormalities including eosinophilia, monocytosis and leucopenia. In non-Hodgkin's lymphoma, malignant cells may circulate in the blood (p. 194).

Platelet and blood coagulation abnormalities

Patients with malignant disease may show either thrombocytosis or thrombocytopenia. Disseminated tumours, particularly mucin-secreting adenocarcinomas, are associated with disseminated intravascular coagulation (DIC; p. 298) and generalized haemostatic failure. Activation of fibrinolysis occurs in some patients with carcinoma of the prostate. Occasional patients with malignant disease have spontaneous bruising or bleeding caused by an acquired inhibitor of one or other coagulation factor, most frequently factor VIII, or to a paraprotein interfering with platelet function.

Cancer patients have a high incidence (estimated at 15%) of venous thromboembolism. This is increased by surgery and some drugs, most recently thalidomide. It is most common in ovarian, brain, pancreatic and colon cancers. It may be difficult to manage with oral anticoagulation because of bleeding, interruptions with chemotherapy and thrombocytopenia, anorexia or vomiting. Liver disease and drug interactions can cause further complications so daily low molecular weight heparin injections may be preferable to warfarin.

Rheumatoid arthritis (and other connective tissue disorders)

In patients with rheumatoid arthritis, the anaemia of chronic disorders is proportional to the severity of the disease. It is complicated in some patients by

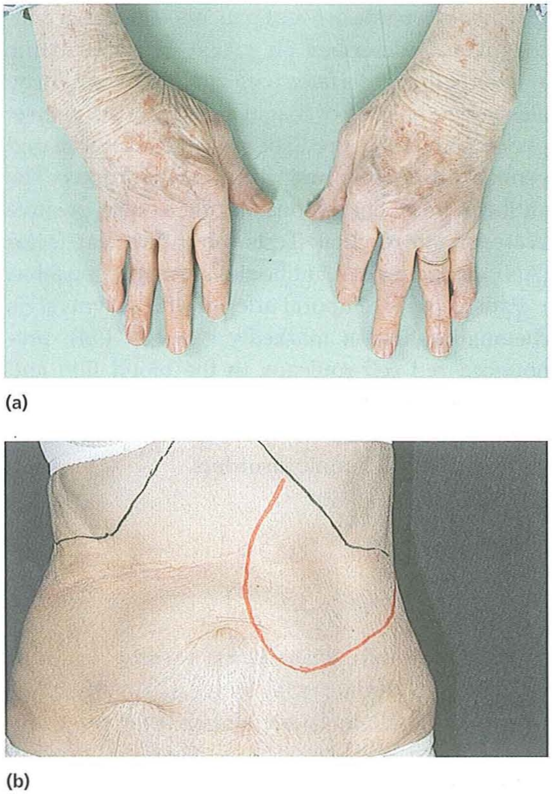


Fig. 26.3 Felty's syndrome: **(a)** the typical deformities of rheumatoid arthritis of the hand and **(b)** splenomegaly.

iron deficiency caused by gastrointestinal bleeding related to therapy with salicylates, non-steroidal anti-inflammatory agents or corticosteroids. Bleeding into inflamed joints may also be a factor. Marrow hypoplasia may follow therapy with gold. In Felty's syndrome, splenomegaly is associated with neutropenia (Fig. 26.3). Anaemia and thrombocytopenia may also be present. In systemic lupus erythematosus (SLE) there may be anaemia of chronic disorders and 50% of patients are leucopenic with reduced neutrophil and lymphocyte counts often associated with circulating immune complexes. Renal impairment and drug-induced gastrointestinal blood loss also contribute to the anaemia. Autoimmune haemolytic anaemia (typically with immunoglobulin G (IgG) and the C3 component of complement on the surface of the red cells) occurs in 5% of patients and may be the presenting feature of the syndrome. There may be autoimmune

thrombocytopenia in 5% of patients. The lupus anticoagulant is described on p. 300. This circulating anticardiolipin interferes with blood coagulation by altering the binding of coagulation factors to platelet phospholipid and predisposes to both arterial and venous thrombosis and recurrent abortions. The antibody may be responsible for a false positive Wassermann reaction. Tests for antinuclear factor (ANF) and anti-DNA antibodies are usually positive.

Patients with temporal arteritis and polymyalgia rheumatica have a markedly elevated ESR, pronounced red cell rouleaux in the blood film and a polyclonal immunoglobulin response. These and other collagen vascular disorders are associated with anaemia of chronic disorders.

Renal failure

Anaemia

A normochromic anaemia is present in most patients with chronic renal failure. Generally, there is a 2 g/dL fall in haemoglobin level for every 10 mmol/L rise in blood urea. There is impaired red cell production as a result of defective erythropoietin secretion (see Fig. 2.5). Uraemic serum has also been shown to contain factors that inhibit proliferation of erythroid progenitors but, in view of the excellent response to erythropoietin in most patients, the clinical relevance of these is doubtful. Variable shortening of red cell lifespan occurs and in severe uraemia the red cells show abnormalities including spicules (spurs) and 'burr' cells (Fig. 26.4). Increased red cell 2,3-diphosphoglycerate (2,3-DPG) levels in response to the anaemia and hyperphosphataemia result in decreased oxygen affinity and a shift of the haemoglobin oxygen dissociation curve to the right (p. 18), which is augmented by uraemic acidosis. The patient's symptoms are therefore relatively mild for the degree of anaemia.

Other factors may complicate the anaemia of chronic renal failure (Table 26.3): the anaemia of chronic disorders, iron deficiency from blood loss during dialysis or caused by bleeding because of defective platelet function, and folate deficiency in some chronic dialysis patients. Aluminium excess in patients on chronic dialysis also inhibits erythropoiesis. Patients with polycystic kidneys usually have retained erythropoietin production and may have less severe anaemia for the degree of renal failure.

Treatment

Erythropoietin therapy has been found to correct the anaemia in patients on dialysis or in chronic renal failure, providing that iron and folate deficiency, aluminium excess and infections have been corrected. The dosage of erythropoietin usually required is 50–150 units/kg three times a week intravenously or by subcutaneous infusion. The response is faster after intravenous administration, but greater with the subcutaneous route.

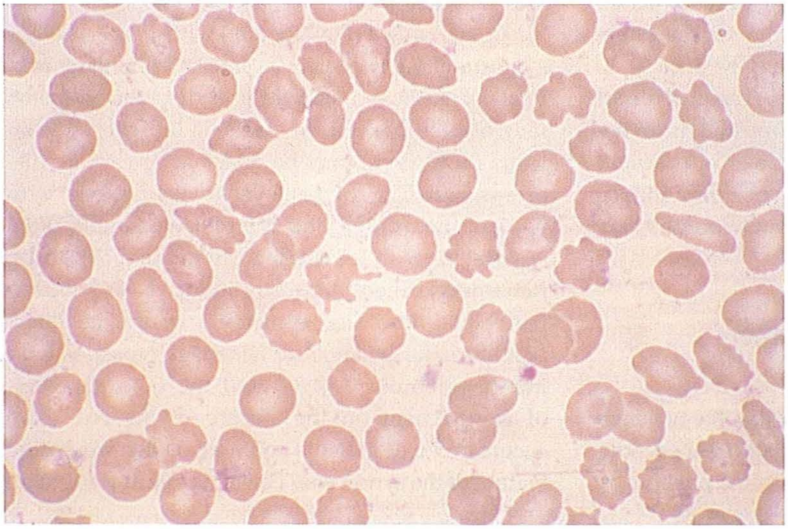


Fig. 26.4 Peripheral blood film in chronic renal failure showing red cell acanthocytosis and numerous 'burr' cells.

Table 26.3 Haematological abnormalities in renal failure.

Anaemia
Reduced erythropoietin production
Aluminium excess in dialysis patients
Anaemia of chronic disorders
Iron deficiency
blood loss (e.g. dialysis, venesection, defective platelet function)
Folate deficiency
chronic haemodialysis without replacement therapy
Abnormal platelet function
Thrombocytopenia
Immune complex-mediated (e.g. systemic lupus erythematosus, polyarteritis nodosa)
Some cases of acute nephritis and following allograft
Haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura
Thrombosis
Some cases of the nephrotic syndrome
Polycythaemia
In renal allograft recipients
Rarely in renal cell carcinoma, cysts, arterial disease

Maintenance by 75 units/kg/week subcutaneously is typical. Complications of therapy have been initial transient flu-like symptoms, hypertension, clotting of the dialysis lines and, rarely, fits. A poor response to erythropoietin suggests iron or folate deficiency, infection, aluminium toxicity or hyperparathyroidism.

Platelet and coagulation abnormalities

A bleeding tendency with purpura, gastrointestinal or uterine bleeding occurs in 30–50% of patients with chronic renal failure and is marked in patients with acute renal failure. The bleeding is out of proportion to the degree of thrombocytopenia and has been associated with abnormal platelet or vascular function, which can be reversed by dialysis. Correction of the anaemia with erythropoietin also improves the bleeding tendency. Immune complex-mediated thrombocytopenia occurs in some patients with acute nephritis, SLE and polyarteritis nodosa and also following renal allografts. Renal allografts may also lead to polycythaemia in 10–15% of patients.

The haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura are discussed on p. 284. Patients with the nephrotic syndrome have an increased risk of venous thrombosis.

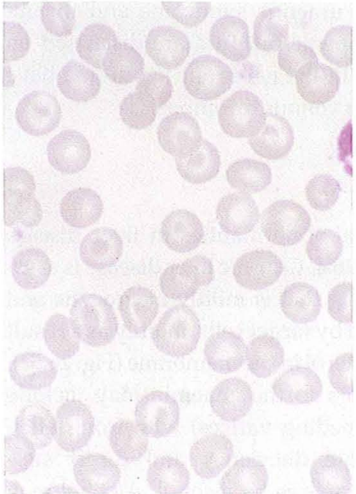
Liver disease

The haematological abnormalities in liver disease are listed in Table 26.4. Chronic liver disease is associated with anaemia that is mildly macrocytic and often accompanied by target cells, mainly as a result of increased cholesterol in the membrane (Fig. 26.5a). Contributing factors to the anaemia may include blood loss (e.g. bleeding varices) with iron deficiency, dietary folate deficiency and direct suppression of haemopoiesis by alcohol. Alcohol may have an inhibiting effect on folate metabolism and is occasionally associated with (ring) sideroblastic changes which disappear when alcohol is withdrawn.

Haemolytic anaemia may occur in patients with alcohol intoxication (Zieve's syndrome) (Fig. 26.5b) and in Wilson's disease (caused by copper oxidation of red cell membranes) and autoimmune haemolytic anaemia is found in some patients with

Table 26.4 Haematological abnormalities in liver disease.

Liver failure ± obstructive jaundice ± portal hypertension
Refractory anaemia—usually mildly macrocytic, often with target cells; may be associated with:
Blood loss and iron deficiency
Alcohol (± ring sideroblastic change)
Folate deficiency
Haemolysis (e.g. Zieve's syndrome, Wilson's disease, immune hypersplenism from portal hypertension)
Bleeding tendency
Deficiency of vitamin K-dependent factors; also of factor V and fibrinogen
Thrombocytopenia hypersplenism, immune platelet function defects
Functional abnormalities of fibrinogen
Increased fibrinolysis
Portal hypertension—haemorrhage from varices
Viral hepatitis
Aplastic anaemia
Tumours
Polycythaemia
Neutrophil leucocytosis and leukaemoid reactions


(a)

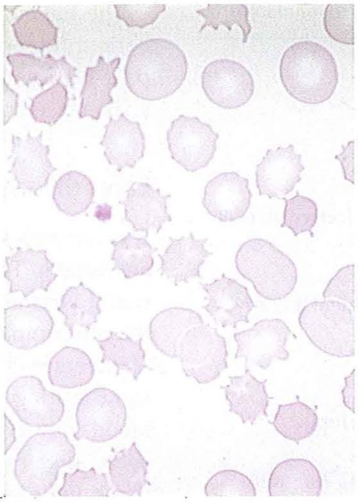

(b)

Fig. 26.5 Liver disease: peripheral blood film showing: **(a)** macrocytosis and target cells; and **(b)** marked acanthocytosis in Zieve's syndrome.

chronic immune hepatitis. Haemolysis may also occur in end-stage liver disease because of abnormal red cell membranes resulting from lipid changes. Viral hepatitis (usually non-A, non-B, non-C) is associated with aplastic anaemia.

The acquired coagulation abnormalities associated with liver disease are described on p. 297. There are deficiencies of vitamin K-dependent factors (II, VII, IX and X) and, in severe disease, of factor V and fibrinogen. Thrombocytopenia may occur from hypersplenism or from immune complex-mediated platelet destruction. Abnormalities of platelet function may also be present. Dysfibrinogenaemia with abnormal fibrin polymerization may occur as a result of excess sialic acid in the fibrinogen molecules. A consumptive coagulopathy may be superimposed. These haemostatic defects may contribute to major blood loss from bleeding varices caused by portal hypertension.

Hypothyroidism

A moderate anaemia is usual and may be caused by lack of thyroxine. T_3 and T_4 potentiate the action of erythropoietin. There is also a reduced oxygen need and thus reduced erythropoietin secretion. The anaemia is often macrocytic and the mean corpuscular volume (MCV) falls with thyroxine therapy. Autoimmune thyroid disease, especially myxoedema or Hashimoto's disease, is associated with pernicious anaemia. Iron deficiency may also be present, particularly in women with menorrhagia.

Infections

Haematological abnormality is usually present in patients with infections of all types (Table 26.5). The effect of inflammation as a prothrombotic stimulus is also discussed on p. 308.

Bacterial infections

Acute bacterial infections are the most common cause of neutrophil leucocytosis. Toxic granulation, Döhle bodies and metamyelocytes may be present in the blood. Leukaemoid reactions with a white cell count $>50 \times 10^9$/L and granulocyte precursors in the blood may occur in severe infections, particularly in infants and young children. The neutrophil alkaline phosphatase (NAP) score is raised in contrast to the low NAP score in chronic myeloid leukaemia. Mild anaemia is common if the infection is prolonged. Severe haemolytic anaemia occurs in bacterial septicaemias, particularly those caused by Gram-negative organisms, where there is usually associated DIC (p. 298). DIC dominates the clinical picture with certain infections (e.g. bacterial meningitis). The acute phase response to infections is accompanied by a rise in coagulation factors

Table 26.5 Blood abnormalities associated with infections.

Haematological abnormality	Infection associated
Anaemia	
Anaemia of chronic disorders	Chronic infections especially tuberculosis
Aplastic anaemia	Viral hepatitis
Transient red cell aplasia	Human parvovirus
Marrow fibrosis	Tuberculosis
Immune haemolytic anaemia	Infectious mononucleosis, *Mycoplasma pneumoniae*
Direct red cell damage or microangiopathic	Bacterial septicaemia (associated DIC), *Clostridium perfringens*, malaria, bartonellosis
	Viruses—haemolytic uraemic syndrome and TTP
Hypersplenism	Chronic malaria, tropical splenomegaly syndrome, leishmaniasis, schistosomiasis
White cell changes	
Neutrophil leucocytosis	Acute bacterial infections
Leukaemoid reactions	Severe bacterial infections particularly in infants
	Tuberculosis
Eosinophilia	Parasitic diseases (e.g. hookworm, filariasis, schistosomiasis, trichinosis)
	Recovery from acute infections
Monocytosis	Chronic bacterial infections: tuberculosis, brucellosis, bacterial endocarditis, typhoid
Neutropenia	Viral infections—HIV, hepatitis, influenza
	Fulminant bacterial infections (e.g. typhoid, miliary tuberculosis)
Lymphocytosis	Infectious mononucleosis, toxoplasmosis, cytomegalovirus, rubella, viral hepatitis, pertussis, tuberculosis, brucellosis
Lymphopenia	HIV infection
	Legionella pneumonophilia
Thrombocytopenia	
Megakaryocytic depression, immune complex-mediated and direct interaction with platelets	Acute viral infections particularly in children (e.g. measles, varicella, rubella, malaria, severe bacterial infection)
Prothrombotic state	All with prolonged inflammation

DIC, disseminated intravascular coagulation; HIV, human immunodeficiency virus; TTP, thrombotic thrombocytopenic purpura.

and a fall in natural anticoagulants. Recombinant human activated protein C reduces the death rate of severe DIC.

Clostridium perfringens organisms produce an α toxin, a lecithinase acting directly on the circulating red cells (Fig. 26.6). Haemolysis in bartonellosis (Oroya fever) is caused by direct red cell infection. With severe acute bacterial infections there may be thrombocytopenia. *Mycoplasma pneumoniae* infections are associated with autoimmune haemolytic anaemia of the 'cold' type (p. 67).

Chronic bacterial infections are associated with the anaemia of chronic disorders. In tuberculosis, additional factors in the pathogenesis of anaemia include marrow replacement and fibrosis associated with miliary disease and reactions to anti-tuberculous therapy (e.g. isoniazid is a pyridoxine antagonist and may cause sideroblastic anaemia). Disseminated tuberculosis is associated with leukaemoid reactions and patients with involvement of bone marrow may show leucoerythroblastic changes in the peripheral blood film.

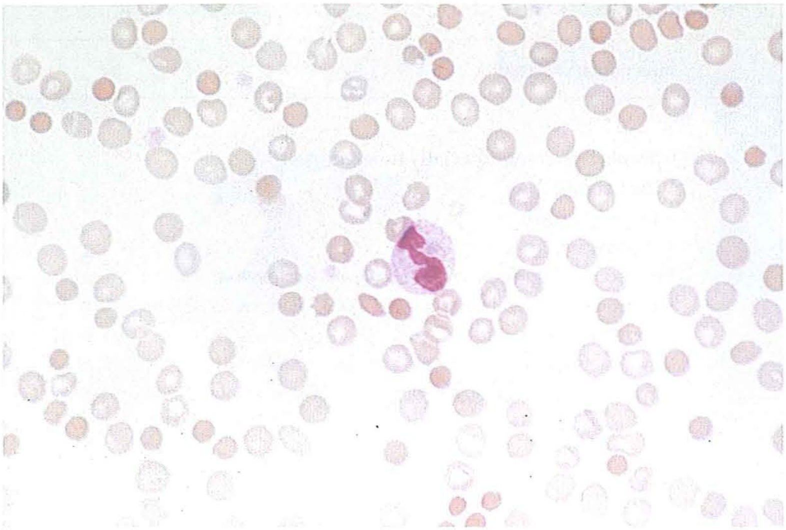


Fig. 26.6 Peripheral blood film in a patient with haemolytic anaemia in clostridial septicaemia showing red cell contraction and spherocytosis.

Viral infections

Acute viral diseases are often associated with a mild anaemia. An immune haemolytic anaemia with an anti-i autoantibody is associated with infectious mononucleosis (p. 68). Viral infections, as well as syphilis, have been associated with paroxysmal cold haemoglobinuria (p. 70). Viruses have also been linked to the pathogenesis of the haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura (pp. 284 and 285) and the haemophagocytic syndrome (p. 106). Aplastic anaemia may occur with viral A or more usually non-A, non-B, non-C hepatitis. Transient red cell aplasia is associated with human parvovirus infection and this may result in severe anaemia in patients with a haemolytic anaemia because of the shortened red cell survival (e.g. in hereditary spherocytosis or sickle cell disease; p. 247).

Acute thrombocytopenia is not uncommon in rubella, morbilli and varicella infections. Rubella and cytomegalovirus (CMV) infections may cause a reactive lymphocytosis similar to that found in infectious mononucleosis. CMV may be responsible for a post-transfusion mononucleosis-like syndrome, CMV being transmitted by leucocytes. CMV infections in infants are associated with massive hepatosplenomegaly. In bone marrow transplant recipients or other immunosuppressed patients, CMV infections may cause pancytopenia as well as other severe disorders (e.g. pneumonitis or hepatitis; p. 258).

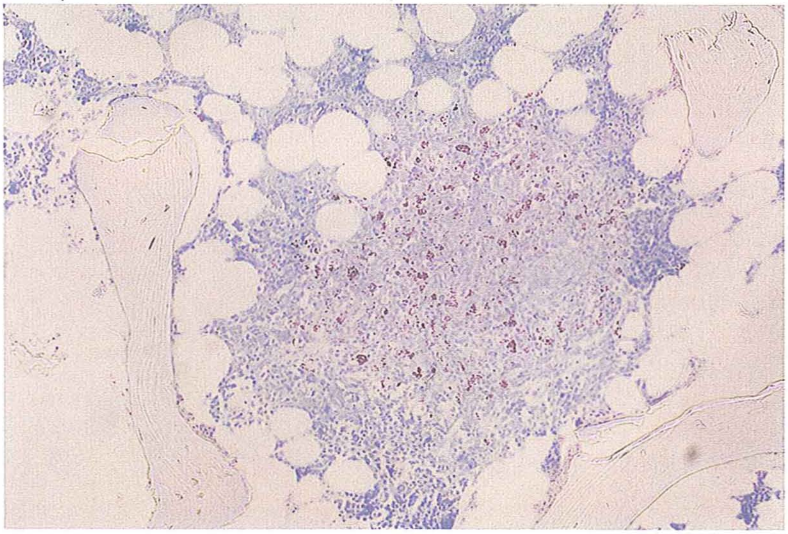
HIV infection

This is associated with a wide range of haematological changes. These are caused by marrow defects and immune cytopenias directly resulting from HIV infection, the effects of opportunistic infections or lymphoma and the side-effects of drugs used to treat HIV itself or drugs for the complicating infection or lymphoma.

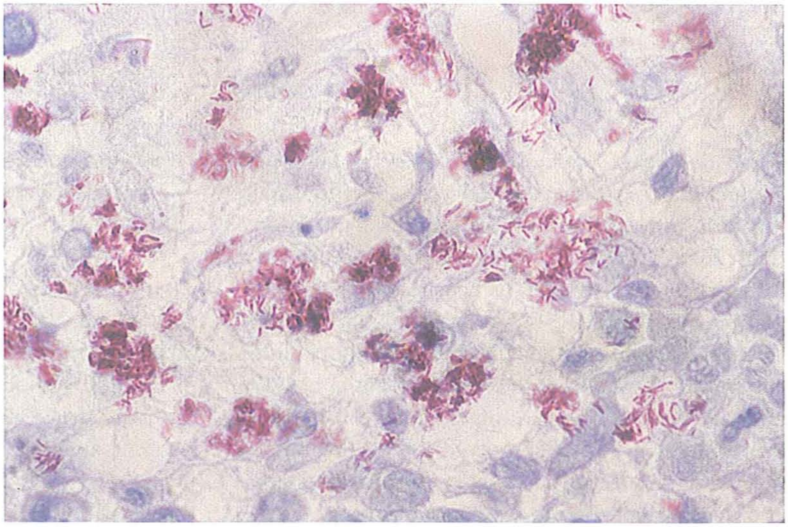
Thrombocytopenia and neutropenia may be immune or secondary to marrow dysfunction. The marrow may be hypercellular with prominent plasma cells and lymphocytes, normocellular, hypocellular or fibrotic. Dysplastic features are common, with ineffective thrombopoiesis or granulocyte formation accounting at least in part for the cytopenia. The myelodysplasia does not show the chromosome abnormalities found in classic myelodysplasia and does not appear to be pre-leukaemic. Thrombocytopenia is treated if necessary by corticosteroids, high-dose gammaglobulin infusions or by other therapies for immune thrombocytopenia (p. 281), or by antiretroviral therapy.

Anaemia is also common, like the other cytopenias, and more severe as the disease progresses. It is usually multifactorial in origin—the anaemia of chronic disorders, marrow dysplasia and drug therapy, especially zidovudine. Serum vitamin B_{12} is often low, most likely because of intestinal malabsorption, but the anaemia does not respond to vitamin B_{12} therapy. Blood transfusions or recombinant erythropoietin injections are needed.

Increased plasma cells in the marrow and polyclonal increase in immunoglobulins are frequent. A paraprotein is present in 5–10% but appears benign. Non-Hodgkin's lymphoma, in over 90% high grade, both systemically and in the central nervous system, occurs in HIV infected individuals with over 100 times the frequency expected in the general population. Diffuse large B-cell lymphomas are most common, with 20% confined to the central nervous system. Approximately one-third are Burkitt's lymphoma. Hodgkin's disease, usually of poor prognosis type, is also increased in frequency. EBV infection appears to underlie this as well as Burkitt's lymphoma. Treatment of lymphomas in the setting of HIV is with standard regimens. Continuation of antiretroviral therapy exaggerates the tendency to cytopenia induced by chemotherapy so prophylaxis against opportunistic infections is important. The bone marrow may indeed reveal the presence of opportunistic infection (Fig. 26.7a,b).

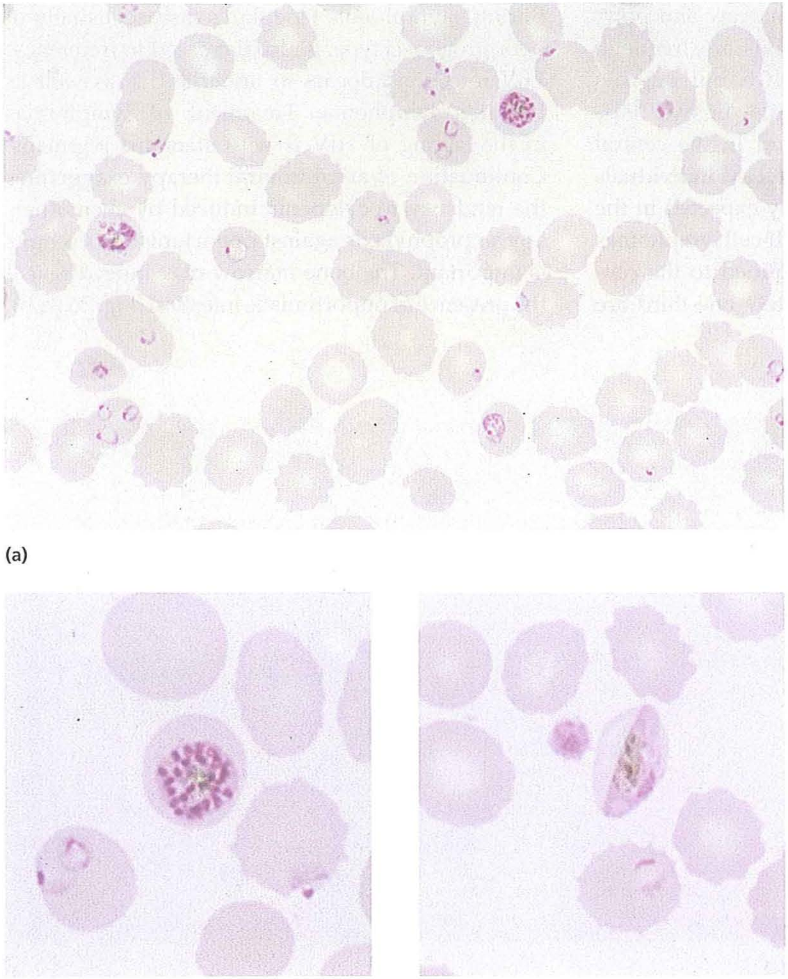


(a)



(b)

Fig. 26.7 Human immunodeficiency virus (HIV) infection: bone marrow trephine biopsy. **(a)** Granuloma showing positivity with Ziehl-Nielsen stain. **(b)** Higher power shows large numbers of acid-fast bacilli



(a)

(b) (c)

Fig. 26.8 Malaria: peripheral blood in severe *Plasmodium falciparum* infection showing: **(a)** many ring forms and a meront; and at higher magnification: **(b)** a meront; and **(c)** a gametocyte.

Malaria

Some degree of haemolysis is seen in all types of malarial infection. The most severe abnormalities are found in *Plasmodium falciparum* infections (Fig. 26.8). In the worst cases, DIC occurs and intravascular haemolysis is marked with haemoglobinuria. This may be associated with quinine therapy ('blackwater fever'). Thrombocytopenia is commonly found in acute malaria. Patients with chronic malaria have an anaemia of chronic disorders; hypersplenism may contribute to the anaemia and result in moderate thrombocytopenia and neutropenia. Tropical splenomegaly (p. 125) is probably a chronic immune reaction to malaria. Dyserythropoiesis in the marrow, folate deficiency and protein-calorie malnutrition may contribute to the anaemia.

Toxoplasmosis

Toxoplasmosis in children and adults is associated with lymphadenopathy and large numbers of atypical lymphocytes in the blood. Congenital disease may be confused with hydrops fetalis in a severely anaemic hydropic infant with gross hepatosplenomegaly, thrombocytopenia or a leucoerythroblastic blood film.

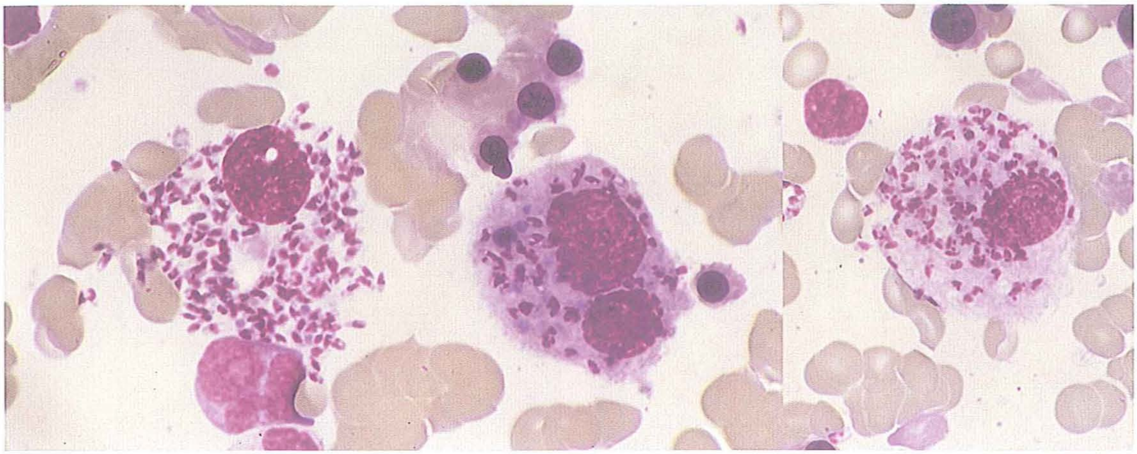


Fig. 26.9 Kala-azar: bone marrow aspirates showing macrophages containing Leishman–Donovan bodies.

Kala-azar (visceral leishmaniasis)

The visceral form of leishmaniasis is associated with pancytopenia, hepatosplenomegaly and lymphadenopathy. Bone marrow or splenic aspirates may show large numbers of parasitized macrophages (Fig. 26.9).

Other parasitic diseases

In the acute phase of both African and South American trypanosomiasis, organisms are found in the peripheral blood (Fig. 26.10). Microfilariae of bancroftian filariasis and loiasis are also detected during blood film examination (Fig. 26.11). In chronic schistosomiasis, hypersplenism follows the splenic enlargement associated with portal hypertension. In many parasitic diseases there is eosinophilia (Table 26.5).

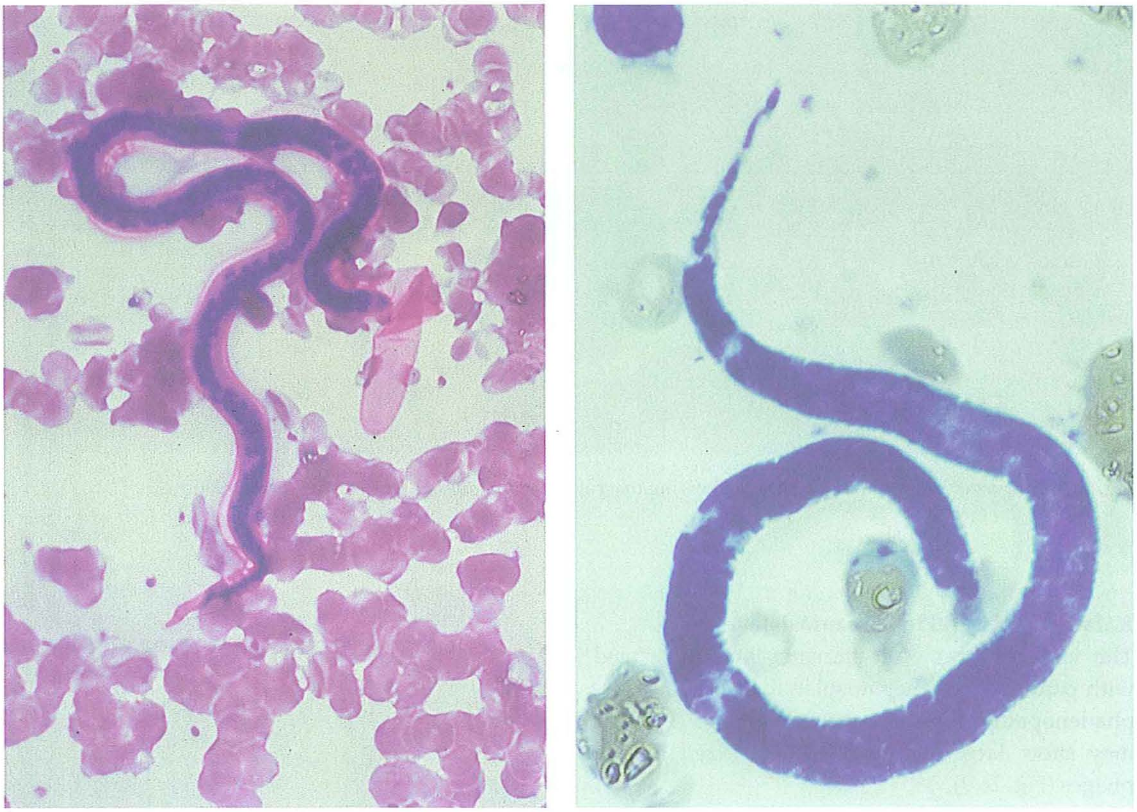
Inborn errors of metabolism

Gaucher's, Tay–Sachs and Niemann–Pick diseases all result from hereditary deficiency of the enzymes required for glycolipid breakdown.

Gaucher's disease

Gaucher's disease is an uncommon autosomal recessive disorder characterized by an accumulation of glucosylceramide in the lysosomes of

Fig. 26.10 African trypanosomiasis: blood film showing *Trypanosoma brucei*.



(a) (b)

Fig. 26.11 Peripheral blood films showing microfilariae of: **(a)** *Wuchereria bancrofti*; and **(b)** *Loa loa*.

reticuloendothelial cells as a result of deficiency of glucocerebrosidase (Fig. 26.12). Three types occur: a chronic adult type (type I); an acute infantile neuronopathic type (type II); and a subacute neuronopathic type with onset in childhood or adolescence (type III). Type I is caused by a variety of mutations in the glucocerebrosidase gene, one type of which (a single base pair substitution in codon 444) is particularly common in Ashkenazi Jews and explains the high incidence of the disease in this group. In type I the outstanding physical sign is splenomegaly. Moderate liver enlargement and pingueculae (conjunctival deposits) are other characteristic findings. In many cases, bone deposits cause bone pain and pathological fractures. Expansion of the lower end of the femur may produce the 'Erlenmeyer flask deformity' (Fig. 26.13c).

The clinical manifestations are caused by the accumulation of glucocerebroside-laden macrophages in the spleen, liver and bone marrow (Fig. 26.13a,b,e). Gaucher's disease at all ages is commonly associated with marked anaemia, leucopenia and thrombocytopenia occurring singly or in combination. Polyclonal hypergammaglobulinemia or monoclonal gammopathy are frequent. Diagnosis is made by assay of white cell glucocerebrosidase and DNA analysis. Lysosomal enyzmes, chitotriosidase and acid phosphatase are raised and useful in monitoring therapy. Pulmonary activation-regulated cytokine (PARC), angiotensin-converting enzyme (ACE) and ferritin are also elevated.

Enzyme replacement therapy with glucocerebrosidase made by recombinant technology and given intravenously is very effective in treating the

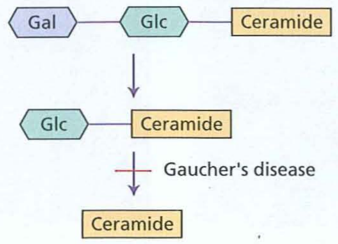


Fig. 26.12 Gaucher's disease results from a deficiency of glucocerebrosidase. Gal, Galactose; Glc, glucose.

disease with shrinkage of spleen, rise in blood count and improved bone structure (Fig. 26.13d,f). An oral drug, miglustat, is useful in mild forms. It reduces the amount of substrate being produced in lysosomes and may be used in combination with the intravenous enzyme. Splenectomy can result in haematological improvement but following this operation there is often increased deposition of cerebroside in extrasplenic tissue, particularly bones. Stem cell transplantation has been carried out successfully in severely affected patients, usually with type II or III disease.

Niemann–Pick disease

Niemann–Pick disease shows certain clinical and pathological similarities to Gaucher's disease. It is caused by a sphingomyelinase deficiency. The majority of patients are infants who die in the first few years of life although occasional patients survive to adult life. Massive hepatosplenomegaly occurs and there is usually lung and nervous system involvement with retarded physical and mental development. A 'cherry-red' spot is commonly seen in the retina of affected infants. Pancytopenia is a regular feature and in marrow aspirates 'foam cells' of similar size to Gaucher cells are seen. Chemical analysis of the tissues reveals that the disorder is caused by an accumulation of sphingomyelin and cholesterol.

Non-specific monitoring of systemic disease

The inflammatory response to tissue injury includes changes in plasma concentrations of proteins known as acute phase proteins. These proteins include fibrinogen and other clotting factors, complement components and CRP (p. 334), haptoglobin, serum amyloid A (SAA) protein, ferritin and others. The rise in these liver-derived proteins is part of a wider response which includes fever, leucocytosis and increased immune reactivity. The acute phase response is mediated by cytokines (e.g. IL-1; see Fig. 7.4) and TNF, released from macrophages and possibly other cells. Patients with chronic disease may show periodic or continuous evidence of the acute phase response depending upon the extent of inflammation. Quantitative measurements of acute phase proteins are valuable indicators of the presence and extent of inflammation and of its response to treatment. When short-term (less than 24 h) changes in the inflammatory response are expected CRP is the test of choice (Table 26.6). Long-term changes in the acute phase proteins are monitored by either the ESR or plasma viscosity. These tests are influenced by plasma proteins which are either slowly responding acute phase reactants (e.g. fibrinogen) or are not acute phase proteins (e.g. immunoglobulins).

Erythrocyte sedimentation rate

This commonly used but non-specific test measures the speed of sedimentation of red cells in plasma over a period of 1 h. The speed is mainly dependent on the plasma concentration of large proteins (e.g. fibrinogen and immunoglobulins). The normal range in men is 1–5 mm/h and in women 5–15 mm/h but there is a progressive increase in old age. The ESR is raised in a wide variety of systemic inflammatory and neoplastic diseases and in pregnancy.

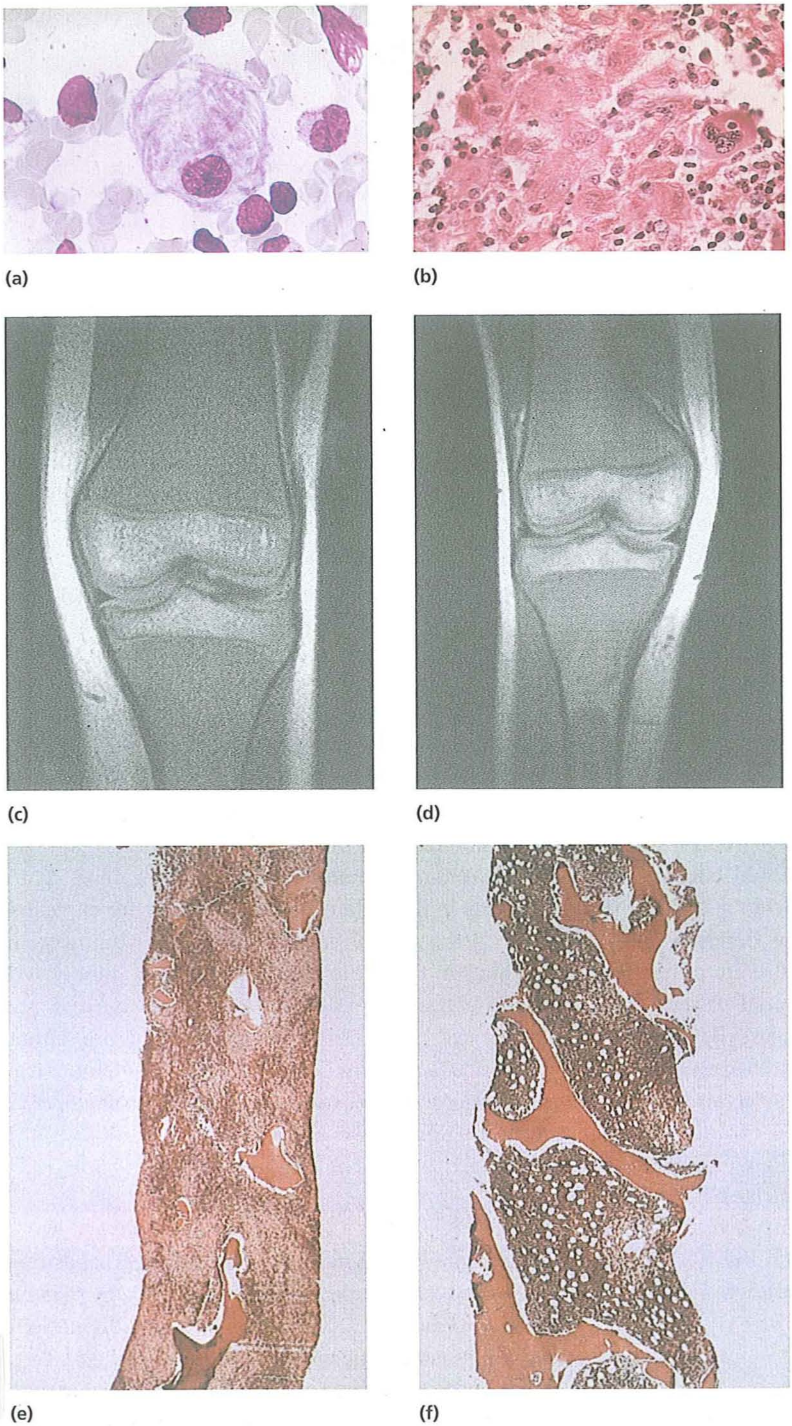


Fig. 26.13 Gaucher's disease: **(a)** bone marrow aspirate—a Gaucher cell with 'fibrillar' cytoplasmic pattern; **(b)** spleen histology—pale clusters of Gaucher cells in the reticuloendothelial cord; **(c)** magnetic resonance imaging (MRI) scan of the left knee of a patient before treatment showing Erlenmeyer flask deformity with expansion of the marrow and thinning of the cortical bone; **(d)** following a year of glucocerebrosidase therapy with subsequent remodelling of bone; and bone marrow trephine biopsy before **(e)** and after **(f)** 2 years of glucocerebrosidase therapy.

Table 26.6 Advantages and disadvantages of the tests used to monitor the acute phase response.

Advantages	Disadvantages
*CRP**	
Specific test of acute phase protein	More than one protein required to measure acute (CRP) and chronic inflammation
Fast response (6 h) to change in disease activity	Costly when assayed in small numbers
High sensitivity—owing to large incremental change	Sophisticated equipment and antisera required
Can be measured on stored serum	
Small sample volumes	
Automated analysis	
ESR and plasma viscosity	
Useful in chronic disease	Not sensitive to acute changes (<24 h)
ESR inexpensive, easy, no electrical power required	Not specific for acute phase response
Plasma viscosity—result obtained quickly (15 min)	Slow to change with alteration in disease activity and insensitive to small changes in activity
Plasma viscosity not affected by anaemia	Fresh samples (<2 h) required for ESR

ESR, erythrocyte sedimentation rate.
* C-reactive protein (CRP) is normally present in low concentrations (<5 mg/L). Levels are not influenced by anaemia, pregnancy or heart failure. During severe acute infection the plasma concentration may rise 100-fold.

It is useful for diagnosing and monitoring temporal arteritis and polymyalgia rheumatica and for monitoring patients with Hodgkin's disease. High values (>100 mm/h) have a 90% predictive value for serious disease including infections, collagen vascular disease or malignancy (particularly myeloma). A raised ESR is associated with marked rouleaux formation of red cells in the peripheral blood film (see Fig. 18.4). Changes in the ESR can be used to monitor the response to therapy.

Lower than expected readings occur in polycythaemia vera because of the high red cell concentration. Higher than expected values may occur in severe anaemia because of the low red cell concentration.

Plasma viscosity

In many laboratories measurement of ESR has been replaced by plasma viscosity measurement. Plasma viscosity is affected by the concentration of plasma proteins of large molecular size, especially those with pronounced axial asymmetry—fibrinogen and some immunoglobulins. Normal values at room temperature are usually in the range of 1.50–1.70 mPa/s. Lower levels are found in neonates because of lower levels of proteins, particularly fibrinogen. Viscosity increases only slightly in the elderly as fibrinogen increases. There is no difference in values between men and women. Other advantages over the ESR test include independence from the effects of anaemia and results that are available within 15 min.

C-reactive protein

Phylogenetically CRP is a crude 'early' immunoglobulin which initiates the inflammatory reaction. CRP–antigen complexes can substitute for antibody in the fixation of Clq and trigger the complement cascade initiating the inflammatory response to antigens or tissue damage, Subsequent binding of C3b on the surface of microorganisms opsonizes them for phagocytosis.

After tissue injury, an increase in CRP, SAA protein and other acute phase reactants may be detected within 6–10 h. Increase in fibrinogen may not occur until 24–48 h following injury. Immunoassays of CRP are now widely used for early detection of acute inflammation or tissue injury and for the monitoring of remission (e.g. response of infection to an antibiotic).

Table 26.6 lists the advantages and disadvantages of the tests used to assess the acute phase response.

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CHAPTER 27

Blood transfusion

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Blood transfusion consists of the 'safe' transfer of blood components (Fig. 27.1) from a donor to a recipient.

Blood donor

This should be voluntary. The measures to protect donors are listed in Table 27.1.

Red cell antigens and blood group antibodies

Approximately 400 red blood cell group antigens have been described. The clinical significance of blood groups in blood transfusion is that individuals who lack a particular blood group antigen may produce antibodies reacting with that antigen which may

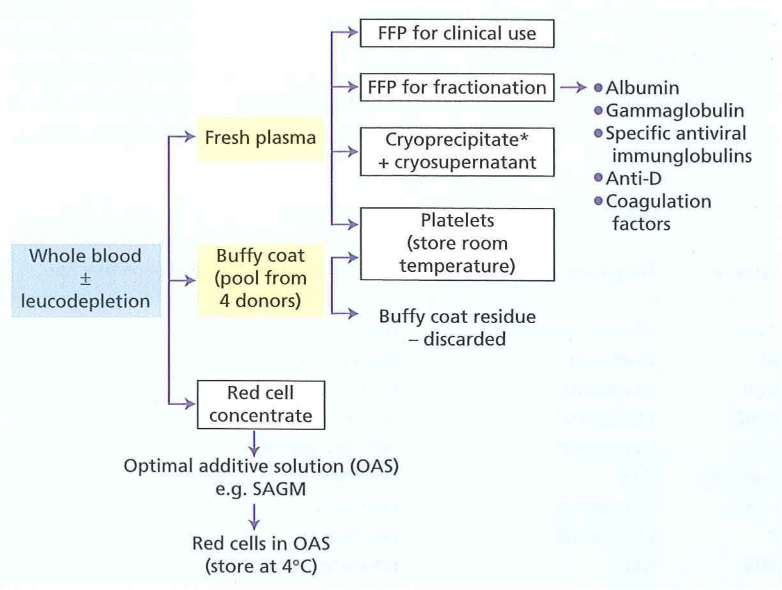


Fig. 27.1 The preparation of blood components from whole blood. FFP, fresh frozen plasma; SAGM, saline-adenine-glucose-mannitol. * Cryoprecipitate is mainly a source of fibrinogen. Cryosupernatant is used for plasma exchange in thrombotic thrombocytopenic purpura.

Table 27.1 Measures to protect the donor.

Age 17–70 years (maximum 60 at first donation)
Weight above 50 kg (7 st 12 lb)
Haemoglobin >13 g/dL for men, >12 g/dL for women
Minimum donation interval of 12 weeks (16 weeks advised) and three donations per year maximum
Pregnant and lactating women excluded because of high iron requirements
Exclusion of those with:
known cardiovascular disease, including hypertension
significant respiratory disorders
epilepsy and other CNS disorders
gastrointestinal disorders with impaired absorption
Insulin-dependent diabetes
Chronic renal disease
Ongoing medical investigation or clinical trials
Exclusion of any donor returning to occupations such as driving bus, plane or train, heavy machine or crane operator, mining, scaffolding, etc. because delayed faint would be dangerous

CNS, central nervous system.

lead to a transfusion reaction. The different blood group antigens vary greatly in their clinical significance with the ABO and Rh (formerly Rhesus) groups being the most important. Some other systems are listed in Table 27.2.

Blood group antibodies

Naturally occurring antibodies occur in the plasma of subjects who lack the corresponding antigen and who have not been transfused or been pregnant. The most important are anti-A and anti-B. They are usually immunoglobulin M (IgM), and react optimally at cold temperatures (4°C) so, although reactive at 37°C, are called cold antibodies.

Immune antibodies develop in response to the introduction—by transfusion or by transplacental passage during pregnancy—of red cells possessing antigens that the subject lacks. These antibodies are commonly IgG, although some IgM antibodies may also develop—usually in the early phase of an immune response. Immune antibodies react optimally at 37°C (warm antibodies). Only IgG antibodies are capable of transplacental passage from mother to fetus. The most important immune antibody is the Rh antibody, anti-D.

ABO system

This consists of three allelic genes: A, B and O. The A and B genes control the synthesis of specific enzymes responsible for the addition of single carbohydrate residues (*N*-acetyl galactosamine for group A and D-galactose for group B) to a basic antigenic glycoprotein or glycolipid with a terminal sugar L-fucose on the red cell, known as the H substance (Fig. 27.2). The O gene is an amorph and does not transform the H substance. Although there are six possible genotypes, the absence of a specific anti-O prevents the serological recognition of more than four phenotypes (Table 27.3). The two major subgroups of A (A_1 and A_2) complicate the issue but are of minor clinical significance. A_2 cells react more

Table 27.2 Clinically important blood group systems.

Systems	Frequency of antibodies	Cause of haemolytic transfusion reaction	Cause of haemolytic disease of newborn
ABO	Almost universal	Yes (common)	Yes (usually mild)
Rh	Common	Yes (common)	Yes
Kell	Occasional	Yes (occasional)	Anaemia not haemolysis
Duffy	Occasional	Yes (occasional)	Yes (occasional)
Kidd	Occasional	Yes (occasional)	Yes (occasional)
Lutheran	Rare	Yes (rare)	No
Lewis	Occasional	Yes (rare)	No
P	Occasional	Yes (rare)	Yes (rare)
MN	Rare	Yes (rare)	Yes (rare)
Li	Rare	Unlikely	No

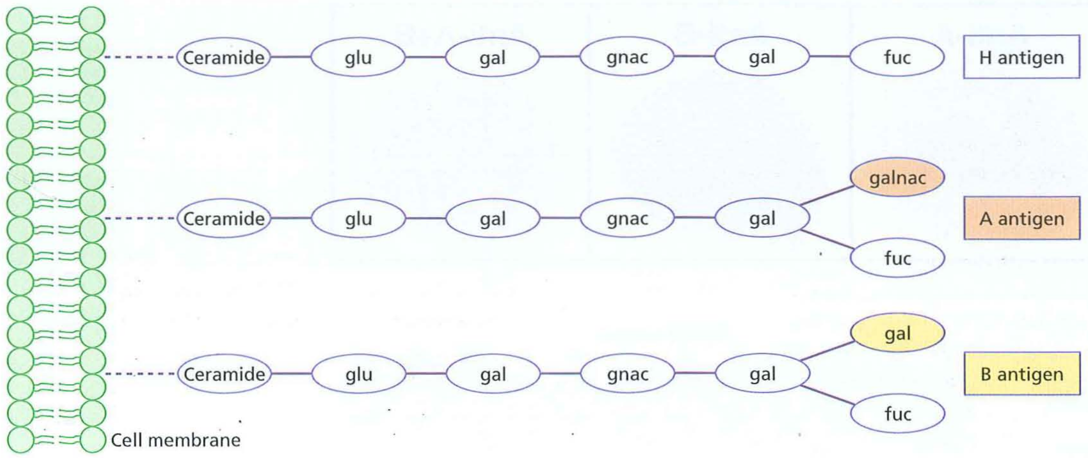


Fig. 27.2 Structure of ABO blood group antigens. Each consists of a chain of sugars attached to lipids or proteins which are an integral part of the cell membrane. The H antigen of the O blood group has a terminal fucose (fuc). The A antigen has an additional *N*-acetyl galactosamine (galnac), and the B antigen has an additional galactose (gal). glu, glucose.

Table 27.3 The ABO blood group system.

Phenotype	Genotype	Antigens	Naturally occurring antibodies	Frequency (UK) (%)
O	OO	O	Anti-A, anti-B	46
A	AA or AO	A	Anti-B	42
B	BB or BO	B	Anti-A	9
AB	AB	AB	None	3

weakly than A_1 cells with anti-A and patients who are A_2B can be wrongly grouped as B.

The A, B and H antigens are present on most body cells including white cells and platelets. In the 80% of the population who possess secretor genes, these antigens are also found in soluble form in secretions and body fluids (e.g. plasma, saliva, semen and sweat).

Naturally occurring antibodies to A and/or B antigens are found in the plasma of subjects whose red cells lack the corresponding antigen (Table 27.3; Fig. 27.3).

Rhesus system

The Rh blood group locus is composed of two related structural genes, *RhD* and *RhCE*, which encode the membrane proteins that carry the D, Cc and Ee antigens. The *RhD* gene may be either present or absent, giving the Rh D+ or Rh D– phenotype, respectively. Alternative RNA splicing from the *RhCE* gene generates two proteins, which encode the C, c, E or e antigens (Fig. 27.4). A shortened nomenclature for Rh phenotype is commonly used (Table 27.4).

Rh antibodies rarely occur naturally; most are immune (i.e. they result from previous transfusion or pregnancy). Anti-D is responsible for most of the clinical problems associated with the system and a simple subdivision of subjects into Rh D+ and Rh D– using anti-D is sufficient for routine clinical purposes. Anti-C, anti-c, anti-E and anti-e are occasionally seen and may cause both transfusion reactions and haemolytic disease of the newborn. Anti-d does not exist. Rh haemolytic disease of the newborn is described in Chapter 28.

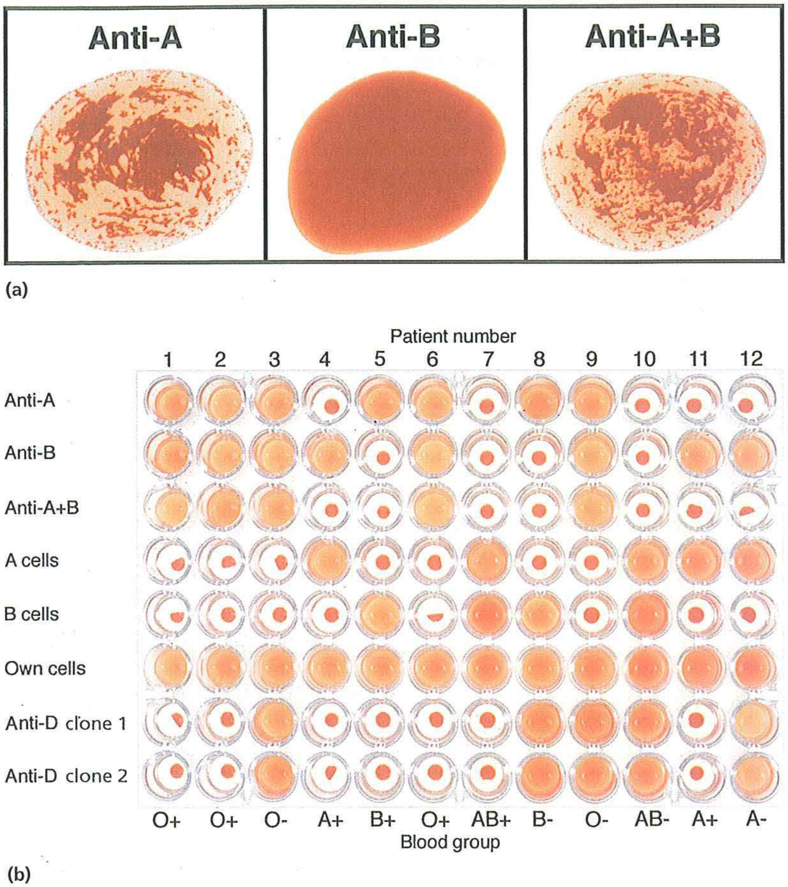


Fig. 27.3 **(a)** The ABO grouping in a group A patient. The red cells suspended in saline agglutinate in the presence of anti-A or anti-A + B (serum from a group O patient). **(b)** Routine grouping in a 96-well microplate. Positive reactions show as sharp agglutinates; in negative reactions the cells are dispersed. Rows 1–3, patient cells against antisera; rows 4–6, patient sera against known cells; rows 7–8, anti-D against patient cells.

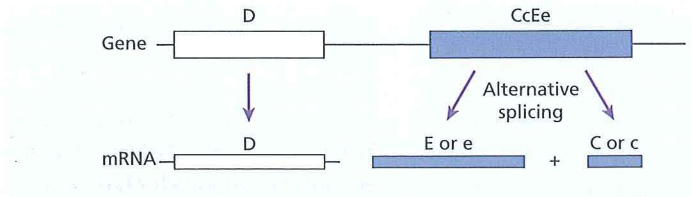


Fig. 27.4 Molecular genetics of the rhesus blood group. The locus consists of two closely linked genes, *RhD* and *RhCcEe*. The *RhD* gene codes for a single protein which contains the RhD antigen whereas *RhCcEe* mRNA undergoes alternative splicing to three transcripts. One of these encodes the E or e antigen whereas the other two (only one is shown) contain the C or c epitope. A polymorphism at position 226 of the *RhCcEe* gene determines the Ee antigen status whereas the C or c antigens are determined by a four amino acid allelic difference. Some individuals do not have an *RhD* gene and are therefore RhD–.

Other blood group systems

Other blood group systems are less frequently of clinical importance. Although naturally occurring antibodies of the P, Lewis and MN system are not uncommon, they usually only react at low temperatures and hence are of no clinical consequence. Immune antibodies against antigens of these systems are detected infrequently. Many of the antigens are of low antigenicity and others (e.g. Kell),

Table 27.4 The most common Rh genotypes in the UK population.

CDE nomenclature	Short symbol	Caucasian frequency (%)	Rh D status
cde/cde	rr	15	Negative
CDe/cde	R_1r	31	Positive
CDe/CDe	R_1R_1	16	Positive
cDE/cde	R_2r	13	Positive
CDe/cDE	R_1R_2	13	Positive
cDE/cDE	R_2R_2	3	Positive
Other genotypes		9	Positive (almost all)

although comparatively immunogenic, are of relatively low frequency and therefore provide few opportunities for isoimmunization except in multiply transfused patients.

Hazards of allogeneic blood transfusion

A large number of measures are taken to protect the recipient (Table 27.5).

Infection

Donor selection and testing of all donations are designed to prevent transmission of diseases (Table 27.6). The main risk is from viruses that have long incubation periods and especially those that are carried for many years by asymptomatic individuals. Some viruses that are transfusion transmissible show cell-associated latency and, if in white cells, can cause infection in the recipient after allogeneic transfusion. Live viruses causing acute infection can be transmitted in the pre-symptomatic viraemic phase if blood is collected during that short period.

Table 27.5 Measures to protect recipient.

Donor selection
Donor deferral/exclusion
Stringent arm cleaning
Microbiological testing of donations (Table 27.6)
Immunohaematological testing of donations
Diversion of first 20–30 mL of blood collected
Leucodepletion of cellular products
Post-collection viral inactivation
Monitoring and testing for bacterial contamination
Pathogen inactivation
Safest possible sources of donor for plasma products

Table 27.6 Microbiological testing in England and Wales.

1 Human immunodeficiency virus (HIV) 1 and 2
2 Hepatitis B virus (HBV)
3 Hepatitis C virus (HCV)
4 Human T-cell leukaemia viruses (HTLV)
5 Cytomegalovirus (CMV)—for immunosuppressed recipients
6 Malaria—antibody screening of potentially exposed donors
7 Chagas' disease—antibody screening of potentially exposed donors
8 Bacteria—all donations tested for antibody to syphilis17p

Individual infections (Table 27.7)

Hepatitis

Donors with a history of hepatitis are deferred for 12 months. If there is a history of jaundice, they can be accepted if markers for HBV and HCV are negative.

Human immunodeficiency virus (HIV)

This can be transmitted by cells or plasma. Male homosexuals, bisexuals, intravenous drug users and prostitutes are excluded, as are their sexual partners and partners of haemophiliacs. Inhabitants of large areas of sub-Saharan Africa and South-East Asia where HIV infection is particularly common are also excluded. Rare transmission occurs when the donor is incubating the infection but is not yet positive for the antigen-antibody test used (window period transmission).

Table 27.7 Infections agents reported to have been transmitted by blood transfusion.

Viruses	
Hepatitis viruses	Hepatitis A virus (HAV)
	Hepatitis B virus (HBV)
	Hepatitis C virus (HCV)
	Hepatitis D virus (HDV) (requires coinfection with HBV)
Retroviruses	Human immunodeficiency virus (HIV) 1 + 2 (+ other subtypes)
	Human T-cell leukaemia virus (HTLV) I + II
Herpes viruses	Human cytomegalovirus (CMV)
	Epstein–Barr virus (EBV)
	Human herpesvirus 8 (HHV-8)
Parvoviruses	Parvovirus B19
Miscellaneous viruses	GBV-C—previously referred to as hepatitis G virus (HGV)
	Transfusion transmitted virus (TTV)
	West Nile virus
Bacteria	
Endogenous	*Treponema pallidum* (syphilis)
	Borrelia burgdorferi (Lyme disease)
	Brucella melitensis (brucellosis)
	Yersinia enterocolitical/Salmonella spp.
Exogenous	Environmental species—staphyloccocal spp./pseudomonas/*Serratia* spp.
Rickettsiae	*Rickettsia rickettsii* (Rocky Mountain spotted fever)
	Coxiella burnettii (Q fever)
Protozoa	
	Plasmodium spp. (malaria)
	Trypanosoma cruzi (Chagas' disease)
	Toxoplasma gondii (toxoplasmosis)
	Babesia microti/divergens (babesiosis)
	Leishmania spp. (leishmaniasis)
Prions	
	New variant Creuzfeldt–Jacob disease (nvCJD)

Human T-cell leukaemias viruses

Human T-cell leukaemias virus type I (HTLVI) is associated with adult T-cell leukaemia or tropical spastic paraperesis. Human T-cell leukaemia virus type II (HLTVII) has no known association with any clinical condition. Screening for both is mandatory in the UK despite the low prevalence, approximately 1 in 50 000 untested donors.

Cytomegalovirus

Post-infusion cytomegalovirus (CMV) infection is usually subclinical but may give an infectious mononucleosis syndrome. Immunosuppressed individuals are at risk of pneumonitis and a potentially fatal disease. These are premature babies (<1500 g), bone marrow and other organ transplant recipients, and pregnant women (the fetus is at risk). For such recipients, CMV negative blood or blood components must be given.

Other infections

Syphilis is more likely to be transmitted by platelets (stored at room temperature) than blood (stored at 4°C). However, all donations are tested. Malarial parasites are viable in blood stored at 4°C, so in endemic areas all recipients are given antimalarial drugs. In non-endemic areas, donors are carefully vetted for travel to tropical areas and in some centres tests for malarial antibodies are performed. Chagas' disease is a significant problem with blood

transfusion in Latin America. **Bacterial infections** resulting from skin commensals are most frequently transmitted by platelets stored for more than 3 days.

Prions The risk of new variant Creuzfeldt–Jacob disease (nvCJD) is considered a threat to blood safety only in the UK. Plasma for fractionation and fresh frozen plasma for infants or children is obtained from the USA. It is unknown how many people could be infected with nvCJD. There are three reports of possible transmission by blood transfusion, so recipients of blood or blood components are now excluded as blood donors in the UK. No screening tests for prions are yet available.

Techniques in blood group serology

The most important technique is based on the agglutination of red blood cells. Saline agglutination is important in detecting IgM antibodies, usually at room temperature and 4°C (e.g. anti-A, anti-B; Fig. 27.3). Addition of colloid to the incubation or proteolytic enzyme treatment of red cells increases the sensitivity of the indirect antiglobulin test, as does low ionic strength saline (LISS). These latter methods can detect a range of IgG antibodies.

The antiglobulin (Coombs') test is a fundamental and widely used test in both blood group serology and general immunology. Antihuman globulin (AHG) is produced in animals following the injection of human globulin, purified complement or specific immunoglobulin (e.g. IgG, IgA or IgM). Monoclonal preparations are also now available. When AHG is added to human red cells coated with immunoglobulin or complement components, agglutination of the red cells indicates a positive test (Fig. 27.5).

The antiglobulin test may be either direct or indirect. The direct antiglobulin test (DAT) is used for detecting antibody or complement on the red cell surface where sensitization has occurred *in vivo*. The AHG reagent is added to washed red cells and agglutination indicates a positive test. A positive test occurs in haemolytic disease of the newborn, autoimmune or drug-induced immune haemolytic anaemia and haemolytic transfusion reactions.

The indirect antiglobulin test (IAT) is used to detect antibodies that have coated the red cells *in vitro*. It is a two-stage procedure: the first step involves the incubation of test red cells with serum; in the second step, the red cells are washed and the AHG reagent is added. Agglutination implies that the original serum contained antibody which has coated the red cells *in vitro*. This test is used as part of the routine antibody screening of the recipient's serum prior to transfusion and for detecting blood group antibodies in a pregnant woman.

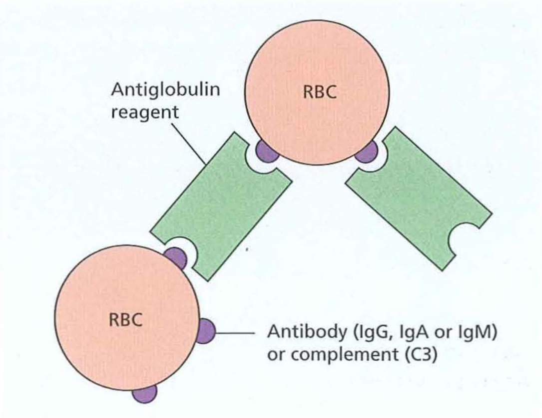


Fig. 27.5 The antiglobulin test for antibody or complement on the surface of red blood cells (RBC). The antihuman globulin (Coombs') reagent may be broad spectrum or specific for immunoglobulin G (IgG), IgM, IgA or complement (C3).

Most of the above methods were originally developed for tube techniques. These were replaced by 96-well microplates but most laboratories now use gel-based technology (Fig. 27.6).

Cross-matching and pre-transfusion tests

A number of steps are taken to ensure that patients receive compatible blood at the time of transfusion.

From the patient

1 The ABO and Rh blood group is determined.

2 Serum is screened for important antibodies by an indirect antiglobulin test on a large panel of antigenically-typed group O red cells.

If a red cell alloantibody is discovered, donor blood is selected lacking the relative antigen. The most likely are Rh D, C, c, E, e and K.

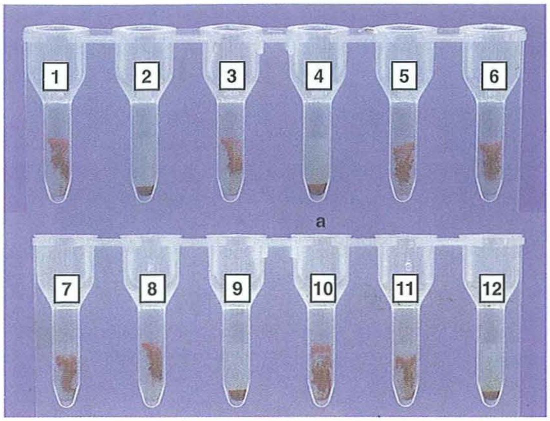


Fig. 27.6 Patient antibody screening using the microcolumn (gel) system: 10 tests with two controls (tube 11 is the positive control and tube 12 the negative control) are shown. The patient's serum is tested against screening cells with known red cell phenotype. Tubes 1, 3, 5–8 and 10 show positive results. The patient's serum contained anti-Fy^a. (Courtesy of Mr G. Hazlehurst)

Table 27.8 Techniques used in compatibility testing. Donor cells tested against recipient serum and agglutination detected visually or microscopically after mixing and incubation at the appropriate temperature.

For detecting clinically significant IgM antibodies
Saline 37°C
For detecting immune antibodies (mainly IgG)
Indirect antiglobulin test at 37°C
Low ionic strength saline at 37°C
Enzyme-treated red cells at 37°C

Ig, immunoglobulin.

From the donor

An appropriate ABO and Rh unit is selected. Donor (blood) testing is described on p. 341.

The cross-match

The techniques that may be used are described in Table 27.8.

Electronic cross-match

In this, a patient has group and antibody screen performed as two separate occasions. If both are negative and no blood has been transfused between the test, ABO and Rh compatible blood is issued directly without wet testing.

Complications of blood transfusion

(Table 27.9)

Haemolytic transfusion reactions

Haemolytic transfusion reactions may be immediate or delayed. Immediate life-threatening reactions associated with massive intravascular haemolysis are the result of complement-activating antibodies of IgM or IgG classes, usually with ABO specificity. Reactions associated with extravascular haemolysis (e.g. immune antibodies of the Rh system which are unable to activate complement) are generally less severe but may still be life-threatening. The cells become coated with IgG and are removed in the reticuloendothelial system. In mild cases, the only signs of a transfusion reaction may be a progressive unexplained anaemia with or without jaundice. In some cases where the pre-transfusion level of an antibody was too low to be detected in a cross-match, a patient may be reimmunized by transfusion of incompatible red cells and this will lead to a delayed transfusion reaction with accelerated clearance of the red cells. There may be rapid appearance of anaemia with mild jaundice.

Clinical features of a major haemolytic transfusion reaction

Haemolytic shock phase This may occur after only a few millilitres of blood have been transfused or up to 1–2 h after the end of the transfusion. Clinical features include urticaria, pain in the lumbar region, flushing, headache, precordial pain, shortness of breath, vomiting, rigours, pyrexia and a fall in blood pressure. If the patient is anaesthetized this shock phase is masked. There is increasing evidence of red cell destruction and haemoglobinuria, jaundice and disseminated intravascular coagulation (DIC) may occur. Moderate leucocytosis (e.g. 15–20 × 10^9/L is usual.

The oliguric phase In some patients with a haemolytic reaction there is renal tubular necrosis with acute renal failure.

Table 27.9 Complications of blood transfusion

Early	Late
Haemolytic reactions: immediate or delayed	Transmission of infection (Table 27.7)
Reactions caused by infected blood	Transfusional iron overload
Allergic reactions to white cells, platelets or proteins	Immune sensitization e.g. to red cells, platelets or Rh D antigen
Pyrogenic reactions (to plasma proteins or caused by HLA antibodies)	Transfusion-associated graft-versus-host disease
Circulatory overload	
Bacterial contamination	
Air embolism	
Thrombophlebitis	
Citrate toxicity	
Hyperkalaemia	
Clotting abnormalities (after massive transfusion)	
TRALI	
Post-transfusion purpura	

CMV, cytomegalovirus; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; TRALI, transfusion-related acute lung injury.

Diuretic phase Fluid and electrolyte imbalance may occur during the recovery from acute renal failure.

Investigation of an immediate transfusion reaction

If a patient develops features suggesting a severe transfusion reaction the transfusion should be stopped and investigations for blood group incompatibility and bacterial contamination of the blood must be initiated.

1 Most severe reactions occur because of clerical errors in the handling of donor or recipient blood specimens. Therefore it must be established that the identity of the recipient (from the patient's wristband) is the same as that on the compatibility label and that this corresponds with the actual unit being transfused.

2 The unit of donor blood and post-transfusion samples of the patient's blood should be sent to the laboratory who will:

(a) repeat the group on pre- and post-transfusion samples and on the donor blood, and repeat the cross-match;

(b) perform a direct antiglobulin test on the post-transfusion sample;

(c) check the plasma for haemoglobinaemia;

(d) perform tests for DIC; and

(e) examine the donor sample directly for evidence of gross bacterial contamination and set up blood cultures from it at 20 and 37°C. If the clinical picture is suggestive of bacterial infection blood cultures must be taken from the patient and broad spectrum intravenous antibodies started.

3 A post-transfusion sample of urine must be examined for haemoglobinuria.

4 Further samples of blood are taken 6 h and/or 24 h after transfusion for a blood count and bilirubin, free haemoglobin and methaemalbumin estimations.

5 In the absence of positive findings, the patient's serum is examined 5–10 days later for red cell or white cell antibodies.

Management of patients with major haemolysis

The principal object of initial therapy is to maintain the blood pressure and renal perfusion. Intravenous dextran, plasma or saline and frusemide are sometimes needed. Hydrocortisone 100 mg intravenously and an antihistamine may help to alleviate shock. In the event of severe shock, support with intravenous adrenaline 1 : 10 000 in small incremental doses may be required. Further compatible

transfusions may be required in severely affected patients. If acute renal failure occurs this is managed in the usual way, if necessary with dialysis until recovery occurs.

Other transfusion reactions

Febrile reactions because of white cell antibodies Human leucocyte antigen (HLA) antibodies (see below and Chapter 21) are usually the result of sensitization by pregnancy or a previous transfusion. They produce rigors, pyrexia and, in severe cases, pulmonary infiltrates. They are minimized by giving leucocyte-depleted (i.e. filtered) packed cells (see below).

Febrile or non-febrile non-haemolytic allergic reactions These are usually caused by hypersensitivity to donor plasma proteins and if severe can result in anaphylactic shock. The clinical features are urticaria, pyrexia and, in severe cases, dyspnoea, facial oedema and rigors. Immediate treatment is with antihistamines and hydrocortisone. Adrenaline is also useful. Washed red cells or frozen red cells may be needed for further transfusions if the majority of plasma-removed blood (e.g. saline, adenine, glucose, mannitol (SAGM) blood) causes reactions.

Post-transfusion circulatory overload The management is that of cardiac failure. These reactions are prevented by a slow transfusion of packed red cells or of the blood component required, accompanied by diuretic therapy.

Transfusion of bacterially contaminated blood This is very rare but may be serious. It can present with circulatory collapse.

Graft-versus-host disease This may occur when live lymphocytes are transfused to an immunocompromised patient. It is prevented by irradiation of the blood product. It is uniformly fatal.

Transfusion related acute lung injury (TRALI) This presents as pulmonary infiltrates with chest symptoms depending on severity. It is caused by positive transfer of leucoagglutins in donor plasma causing endothelial and epithelial injury. Most of the donors are multiparous women. Management is supportive.

Post-transfusion purpura This is a rare problem of severe thrombocytopenia 7–10 days after transfusion of a platelet-containing product, usually red cells. It is caused by an antibody in the recipient (previously transfused or pregnant) anti HPA-1a against a platelet-specific antigen HPA-Ia (PI^{AI}). Both transfused and recipient platelets are destroyed by the immune complexes. It is usually self-limiting but immunoglobulin or plasma exchange may be needed.

Viral transmission Post-transfusion hepatitis may be caused by one of the hepatitis viruses, although CMV and Epstein–Barr virus (EBV) have also been implicated. Post-transfusion hepatitis and HIV infection is seen rarely now because of routine screening of all blood donations.

Other infections Toxoplasmosis, malaria and syphilis may be transmitted by blood transfusion. Transfusion-transmitted nvCJD has probably occurred in three cases in the UK.

Post-transfusional iron overload Repeated red cell transfusions over many years, in the absence of blood loss, cause deposition of iron initially in reticuloendothelial tissue at the rate of 200–250 mg/unit of red cells. After 50 units in adults, and lesser amounts in children, the liver, myocardium and endocrine glands are damaged with clinical consequences. This is a major problem in thalassaemia major and other severe chronic refractory anaemias (Chapter 6).

Reduction of blood product use

In light of transfusion risks, and limited resources, appropriate use of blood component is of ever increasing importance.

Preoperative correction of anaemia (particularly iron deficiency) and cessation of anti-platelet therapies (e.g. aspirin) where possible, together with lower trigger levels for red cell transfusions (7–8 g/dL in most surgical patients) can all help to reduce blood use.

In surgery the use of alternative fluid replacement, intraoperative or post-operative cell salvage, biological alternatives (e.g. erythopoetin, recombinant clotting factors, recombinant activated clotting factor VII (VIIa) or fibrin glue) all may help.

Blood components

A blood donation is taken by an aseptic technique into plastic bags containing an appropriate amount of anticoagulant—usually citrate, phosphate, dextrose (CPD). The citrate anticoagulates the blood by combining with the blood calcium. Three components are made by initial centrifugation of whole blood: red cells, buffy coat and plasma (Fig. 27.1).

Red cells are stored at 4–6°C for up to to 35 days, depending on the preservative. After the first 48 h there is a slow progressive K^+ loss from the red cells into the plasma. In cases where infusion of K^+ could be dangerous, fresh blood should be used (e.g. for exchange transfusion in haemolytic disease of the newborn). During red cell storage there is a fall in 2,3-diphosphoglycerate (2,3-DPG) but after transfusion 2,3-DPG levels return to normal within 24 h. Optimum additive solutions have been developed to increase the shelf life of plasma-depleted red cells by maintaining both adenosine triphosphate (ATP) and 2,3-DPG levels.

Platelets and plasma may also be collected by apharesis.

Leucodepletion

In many countries, including Britain, blood products are now routinely filtered to remove the majority of white cells, a process known as leucodepletion. This is usually performed soon after collection and prior to processing and is more effective than filtration of blood at the bedside. A blood component is defined as leucocyte depleted if there are less than 5×10^6/L white cells present.

Leucodepletion reduces the incidence of febrile transfusion reactions and HLA alloimmunization. It is effective at preventing transmission of CMV infection and in addition should reduce the theoretical possibility of transmission of nvCJD in countries where this has been reported.

Red cells

Packed (plasma-depleted) red cells are the treatment of choice for most transfusions (Fig. 27.7a). In older subjects, a diuretic is often given simultaneously and the infusion should be sufficiently slow to avoid circulatory overload. Iron chelation therapy should be considered with patients on a regular transfusion programme to avoid iron overload.

Recombinant erythropoietin is widely used to reduce transfusion requirements (e.g. in patients on dialysis, cancer patients and myelodysplasia). Factor VIIa can reduce transfusion need in patients with major haemorrhage (e.g. at surgery or after trauma).

Red cell substitutes are under development but have not yet proven clinically valuable. These synthetic oxygen-carrying substitutes are often fluorinated hydrocarbons and stromal-free pyridoxylated and polymerized haemoglobin solutions.

Autologous donation and transfusion

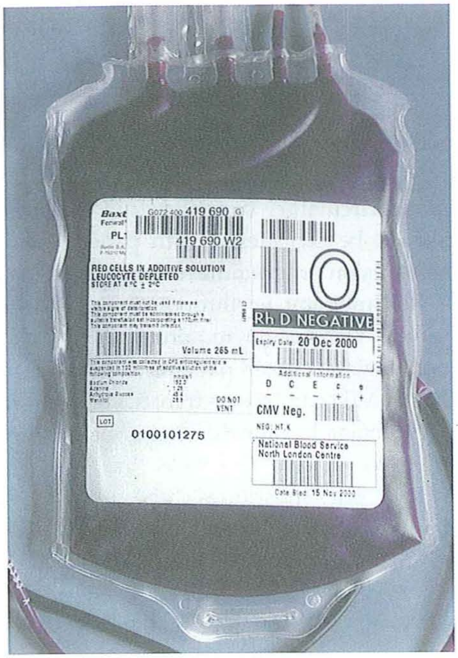
Anxiety over acquired immune deficiency syndrome (AIDS) and other infections has increased the demand for autotransfusion. There are three ways of administering an autologous transfusion:

1 *Predeposit* Blood is taken from the potential recipient in the weeks immediately prior to elective surgery.

2 *Haemodilution* Blood is removed immediately prior to surgery once the patient has been anaesthetized and then reinfused at the end of the operation.

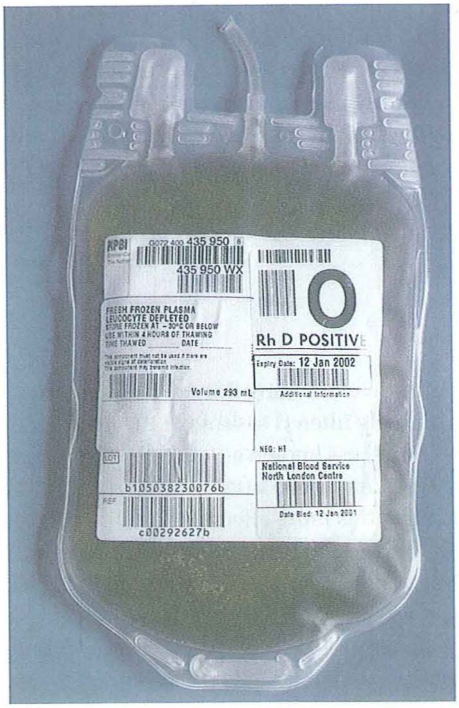
3 *Salvage* Blood lost during the operation is collected during heavy blood loss and then reinfused.

Autotransfusion is the safest form of transfusion with regard to transmission of viral disease though it has a higher risk of bacterial contamination and of clerical errors. The individual involved must be fit enough to donate blood and the predicted operative replacement transfusion should be 2–4 units. Larger replacement transfusions would require blood to be collected over a longer period and red cells stored in the frozen state, which is both labour intensive and expensive. The high cost and initial restriction of its use to patients undergoing elective surgery means that it will benefit only a minor proportion of the total number of blood recipients.



(a)

(b)



(c)

Fig. 27.7 Blood components: **(a)** plasma-depleted red cells; **(b)** platelets; and **(c)** fresh frozen plasma.

Preoperative autotransfusion is largely reserved for those patients with multiple antibodies.

Granulocyte concentrates

These are prepared as buffy coats or on blood cell separators from normal healthy donors or from patients with chronic myeloid leukaemia. They have been used in patients with severe neutropenia ($<0.5 \times 10^9$/L) who are not responding to antibiotic therapy but it is not usually possible to give sufficient amounts. They may transmit CMV infection and must be irradiated to eliminate the risk of causing GVHD.

Platelet concentrates

These are harvested by cell separators or from individual donor units of blood (Fig. 27.7b). They are stored at room temperature. Platelet transfusion is used in patients who are thrombocytopenic or have disordered platelet function and who are actively bleeding (therapeutic use) or are at serious risk of bleeding (prophylactic use).

For prophylaxis, the platelet count should be kept above 5–10 $\times$ 10^9/L unless there are additional risk factors such as sepsis, drug use or coagulation disorders for which the threshold should be higher. For invasive procedures (e.g. liver biopsy or lumbar puncture) the platelet count should be raised to above 50×10^9/L. For brain or eye surgery the count should be $>100 \times 10^9$/L.

Therapeutic use is indicated in bleeding associated with platelet disorders. In massive haemorrhage the count should be kept above 50×10^9/L.

Platelet transfusions should be avoided in autoimmune thrombocytopenic purpura unless there is serious haemorrhage. They are contraindicated in heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome (p. 284).

Refractoriness to platelet transfusions is defined by a poor platelet increment post-transfusion ($<7.5 \times 10^9$/L at 1 h or $<4.5 \times 10^9$/L at 24 h). The causes are either immunological (mostly HLA alloimmunization) or non-immunological (sepsis, hypersplenism, DIC, drugs). Platelets express HLA class I (but not class II) antigens and HLA-matched or cross-match-compatible platelets are needed for patients with HLA antibodies.

Preparations from human plasma

Fresh frozen plasma (FFP)

Rapidly frozen plasma separated from fresh blood is stored at less than –30°C. Frozen plasma is usually prepared from single donor units though pooled products are also available. Its main use is for the replacement of coagulation factors (e.g. when specific concentrates are unavailable) or after massive transfusions, in liver disease and DIC, after cardiopulmonary bypass surgery, to reverse a warfarin effect, and in thrombotic thrombocytopenic purpura (see p. 284). Virally inactivated forms of FFP are now available.

Human albumin solution (4.5%)

It is a useful plasma volume expander when a sustained osmotic effect is required prior to the administration of blood, but it should not be given in excess. It is also used for fluid replacement in patients undergoing plasmapheresis and sometimes for fluid replacement in selected patients with hypoalbuminaemia.

Human albumin solution (20%) (salt-poor albumin)

It may be used in severe hypoalbuminaemia when it is necessary to use a product with minimal electrolyte content. Principal indications for its use are patients with nephrotic syndrome or liver failure.

Cryoprecipitate

This is obtained by thawing FFP at 4°C and contains concentrated factor VIII and fibrinogen. It is stored at less than –30°C or, if lyophylized, at 4–6°C, and was used widely as replacement therapy in haemophilia A and von Willebrand disease before more purified preparations of factor VIII became available. Its main use is in fibrinogen replacement in disseminated intravascular coagulation (DIC) or massive transfusion or hepatic failure.

Freeze-dried factor VIII concentrates

These are also used for treating haemophilia A or von Willebrand disease. The small volume makes them ideal for children, surgical cases, patients at risk from circulatory overload and for those on home treatment. Their use is declining as recombinant forms of factor VIII become widely available.

Freeze-dried factor IX–prothrombin complex concentrates

A number of preparations are available that contain variable amounts of factors II, VII, IX and X. They are mainly used for treating factor IX deficiency (Christmas disease) but are also used in patients with liver disease or in haemorrhage following overdose with oral anti-coagulants or in patients with factor VIII inhibitors. There is a risk of thrombosis.

Protein C concentrate

This is used in severe sepsis with disseminated intravascular coagulation (e.g. meningococcal septicaemia) to reduce thrombosis resulting from depletion of protein C.

Immunoglobulin

Pooled immunoglobulin is a valuable source of antibodies against common viruses. It is used in hypogammaglobulinaemia for protection against viral and bacterial disease. It may also be used in immune thrombocytopenia and other acquired immune disorders (e.g. post-transfusion purpura or alloimmune neonatal thrombocytopenia).

Specific immunoglobulin

This may be obtained from donors with high titres of antibody (e.g. anti-RhD, antihepatitis B, anti-herpes zoster or antirubella).

Acute blood loss

After a single episode of blood loss, there is initial vasoconstriction with a reduction in total blood volume. The plasma volume rapidly expands and the haemoglobin and packed cell volume fall and there is a rise in neutrophils and platelets. The reticulocyte response begins on the second or third day and lasts 8–10 days. The haemoglobin begins to rise by about the seventh day but, if iron stores have become depleted, the haemoglobin may not rise subsequently to normal. Clinical assessment is needed to gauge whether blood transfusion is needed. This is usually unnecessary in adults at losses less than 500 mL unless haemorrhage is continuing and may not be needed with losses of up to 1.5 L. Blood transfusion is not without risks and should not be undertaken lightly. The problems of massive blood loss and massive transfusion are considered on p. 301.

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CHAPTER 28

28 Pregnancy and neonatal haematology

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Haematology of pregnancy

Pregnancy places extreme stresses on the haematological system and an understanding of the physiological changes that result is obligatory in order to interpret any need for therapeutic intervention.

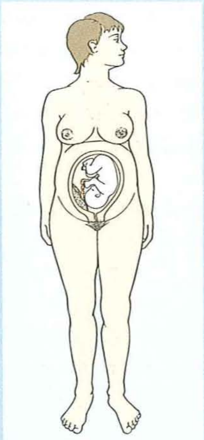
Physiological anaemia

Physiological anaemia is the term often used to describe the fall in haemoglobin (Hb) concentration that occurs during normal pregnancy (Fig. 28.1). Blood plasma volume increases by approximately 1250 mL, or 45%, above normal by the end of gestation and although the red cell mass itself increases by some 25% this still leads to a fall in Hb concentration. Values below 10 g/dL are probably abnormal and require investigation.

Iron deficiency anaemia

Up to 600 mg iron is required for the increase in red cell mass and a further 300 mg for the fetus. Despite an increase in iron absorption, few women avoid depletion of iron reserves by the end of pregnancy.

In uncomplicated pregnancy, the mean corpuscular volume (MCV) typically rises by approximately 4 fL. A fall in red cell MCV is the earliest sign of iron deficiency. Later, the mean corpuscular haemoglobin



1. **Physiological anaemia**
   - 45% rise in blood plasma volume
   - 25% rise in red cell mass
2. **Thrombocytopenia**
   - typically a 10% fall in platelet count
3. **Coagulation**
   - increased coagulation factors
   - reduced fibrinolysis
4. **Increased requirements for erythropoiesis**
   - 2–3 fold increase in folate requirements
   - 900 mg of iron required for mother and fetus

Fig. 28.1 Haematological changes during pregnancy.

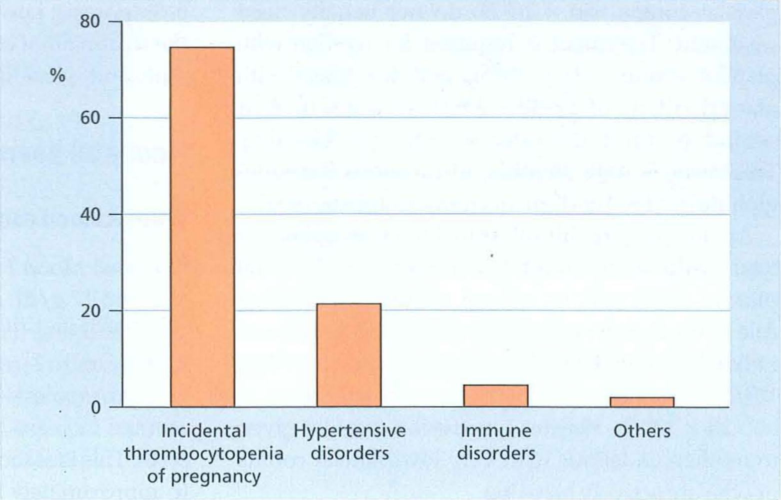


Fig. 28.2 Causes of thrombocytopenia during pregnancy.

(MCH) falls and finally anaemia results. Early iron deficiency is likely if the serum ferritin is below 15 μg/L together with serum iron <10 μmol/L and should be treated with oral iron supplements. The use of routine iron supplementation in pregnancy is debated but iron is probably better avoided until the Hb falls below 10 g/dL or MCV below 82 fL in the third trimester.

Folate deficiency

Folate requirements are increased approximately twofold in pregnancy and serum folate levels fall to approximately half the normal range with a less dramatic fall in red cell folate. In some parts of the world, megaloblastic anaemia during pregnancy is common because of a combination of poor diet and exaggerated folate requirements. Given the protective effect of folate against neural tube defects, folic acid 400 μg/day should be taken periconceptually and throughout pregnancy. Food fortification with folate is now being practised in many countries. Vitamin B_{12} deficiency is rare during pregnancy although serum vitamin B_{12} levels fall to below normal in 20–30% of pregnancies and low values are sometimes the cause of diagnostic confusion.

Thrombocytopenia

The platelet count typically falls by approximately 10% in an uncomplicated pregnancy. In approximately 7% of women this fall is more severe and can result in thrombocytopenia (platelet count $<140 \times 10^9$/L). In over 75% of cases this is mild and of unknown cause, a condition referred to as *incidental thrombocytopenia of pregnancy*. Approximately 21% of cases are secondary to a hypertensive disorder and 4% are associated with immune thrombocytopenic purpura (ITP; Fig. 28.2).

Incidental thrombocytopenia of pregnancy This is a diagnosis of exclusion and is usually detected at the time of delivery. The platelet count is always $>70 \times 10^9$/L and recovers within 6 weeks. No treatment is required and the infant is not affected.

Thrombocytopenia of hypertensive disorders This is variable in severity but the platelet count rarely falls to $<40 \times 10^9$/L. It is more severe when associated with pre-eclampsia and if severe the primary treatment is as rapid delivery as possible. The platelet count falls for a day or two after delivery and then recovers rapidly. The HELLP syndrome (haemolysis, elevated liver enzymes and low platelets) is a subtype of this category.

Idiopathic thrombocytopenic purpura (p. 281) In pregnancy, ITP represents a particular problem, both to the mother and to the fetus, as the antibody crosses the placenta and the fetus may become severely thrombocytopenic.

Like all adults, pregnant women with ITP and

platelet counts >50 × 10^9/L do not usually need treatment. Treatment is required for women with platelet counts <10 × 10^9/L and for those with platelet counts of 10–30 × 10^9/L who are in their second or third trimester or who are bleeding. Treatment is with steroids, intravenous immunoglobulin G (IgG) and splenectomy as appropriate.

At delivery, umbilical vein blood sampling or fetal scalp vein sampling to measure the fetal platelet count may be offered although their exact role is unclear. In general, caesarean section is not indicated when the maternal platelet count is >50 × 10^9/L unless the fetal platelet count is known to be <20 × 10^9/L. Platelet transfusion may be given to mothers in labour with very low platelet counts or who are actively bleeding.

Newborns of mothers with ITP should have a blood count measured for the first 4 days of life as the platelet count may progressively drop. A count greater than 50 × 10^9/L is reassuring. Cerebral ultrasounds may be performed to look for intracranial haemorrhage (ICH). In newborns without evidence of ICH, treatment with intravenous IgG is appropriate if the infant's platelet count is <20 × 10^9/L. Neonates with thrombocytopenia and ICH should be treated with steroids and intravenous IgG therapy.

Haemostasis and thrombosis

Pregnancy leads to a hypercoaguable state with consequent increased risks of thromboembolism and disseminated intravascular coagulation (DIC; p. 298). There is an increase in plasma factors VII, VIII, X and fibrinogen and fibrinolysis is suppressed. These changes last for up to 2 months into the puerperal period and the incidence of thrombosis during this period is increased. There is an association between thrombophilic conditions in the mother and with recurrent fetal loss. This is presumed to result from placental thrombosis and infarction.

Treatment of thrombosis

Warfarin has no role in management. It crosses the placenta and in addition is associated with embryopathy, especially between 6 and 12 weeks' gestation. Heparin does not cross the placenta but a significant side-effect of prolonged use is maternal osteoporosis. Low molecular weight heparin is now the treatment of choice because it can be given once daily and is less likely to cause osteoporosis.

Neonatal haematology

Normal blood count

The cord blood Hb varies between approximately 16.5 and 17 g/dL and is influenced by the timing of cord clamping (Fig. 28.3). The reticulocyte count is initially high (2–6%) but falls to below 0.5% at 1 week as erythropoiesis is suppressed in response to the marked increase in the oxygenation of tissues after birth. This is associated with a progressive fall in Hb to approximately 10–11 g/dL at 8 weeks from which point it recovers to 12.5 g/dL at around 6 months. In the blood film, nucleated red cells will be seen for the first 4 days and for up to 1 week in preterm infants. Numbers are increased in cases of hypoxia, haemorrhage or haemolytic disease of the newborn (HDN). MCV averages 119 fL but falls to adult levels by around 9 weeks. By 1 year, the MCV has fallen to around 70 fL and rises throughout childhood again to reach adult levels at puberty. Preterm infants have a more dramatic fall in Hb to 7–9 g/dL at 8 weeks and are more prone to iron and folate deficiency in the first few months of life. Neutrophils are initially high at birth and fall to plateau at 4 days—from this point on the lymphocyte count is higher than neutrophils throughout childhood.

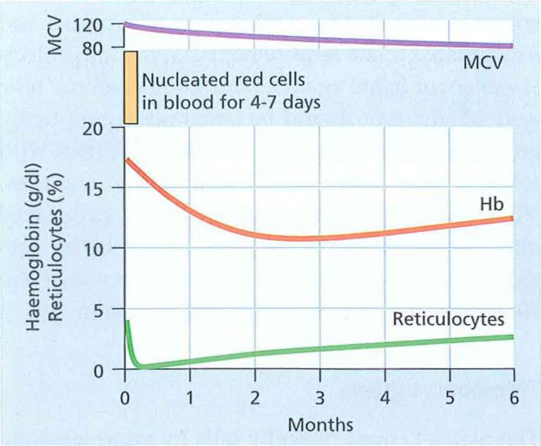


Fig. 28.3 Typical profile of the blood count in the neonatal period.

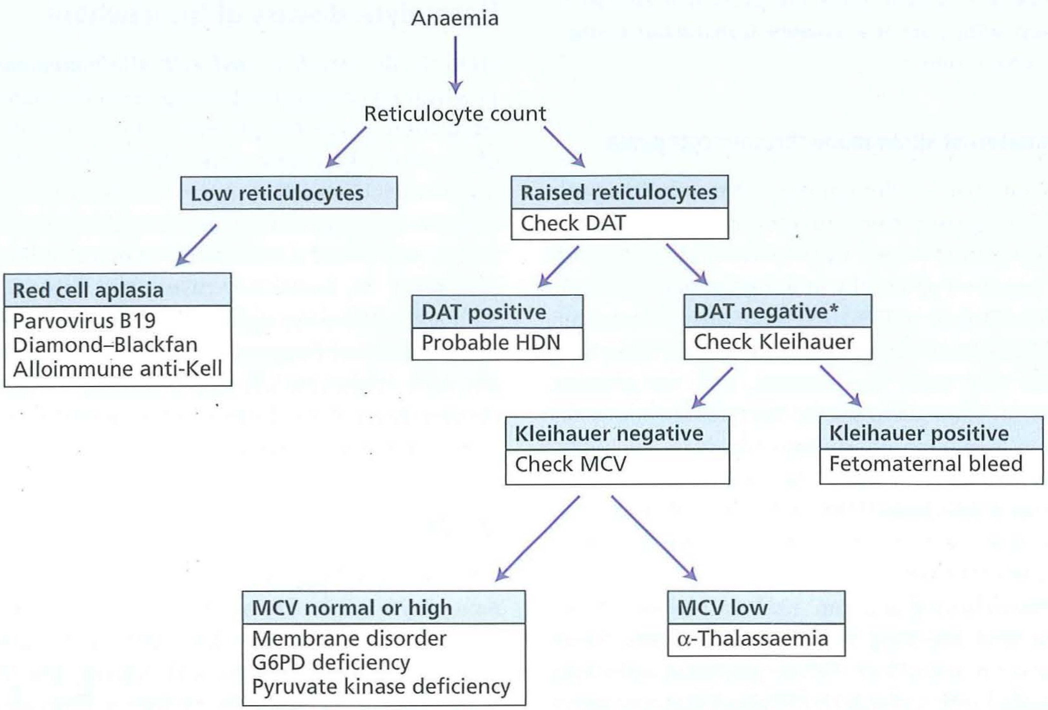


Fig. 28.4 The investigation of neonatal anaemia. * The DAT test may be negative in HDN, e.g. due to ABO incompatibility. DAT, direct antiglobulin test; HDN, haemolytic disease of the newborn; MCV, mean corpuscular volume.

Anaemia in the neonate

This should be considered for Hb <14 g/dL at birth. The clinical significance of anaemia is compounded by the high (70–80%) levels of HbF at birth, as this is less effective than HbA at releasing oxygen to the tissues (p. 17). Causes include the following (Fig. 28.4):

1 *Haemorrhage* Fetomaternal, twin–twin, cord, internal, placenta.

2 *Increased destruction* Haemolysis (immune or non-immune) or infection.

3 *Decreased production* Congenital red cell aplasia, infection (e.g. parvovirus). Anti-Kell causes alloimmune anaemia of the fetus and newborn with decreased erythropoiesis.

Generally, anaemia at birth is usually secondary to immune haemolysis or haemorrhage; non-immune causes of haemolysis appear within 24 h. Impaired red cell production is usually not apparent for at least 3 weeks. Haemolysis is often associated with severe jaundice and the causes include HDN, autoimmune haemolytic anaemia (AIHA) in the mother and congenital disorders of the red cell membrane or metabolism.

Red cell transfusion may be needed for symptomatic anaemia with Hb <10.5 g/dL or a higher threshold if there is severe cardiac or respiratory disease.

Anaemia of prematurity

Premature infants have a more marked fall in Hb after birth and this is termed *physiological anaemia of prematurity*. Features include a slowly falling Hb, normal blood film and reticulocytopenia. It can be minimized by ensuring adequate iron and folate replacement and limiting phlebotomy. Erythropoietin is used in some centres.

Neonatal polycythaemia

This is defined as a venous haematocrit over 0.65 and can occur with twin–twin transfusion, intrauterine growth restriction and maternal hypertension

or diabetes. If symptoms are present it should be treated with partial exchange transfusion using a crystalloid solution.

Fetomaternal alloimmune thrombocytopenia

Fetomaternal alloimmune thrombocytopenia (FMAIT) results from an immunological process similar to that which causes HDN. Fetal platelets that possess a paternally inherited antigen (HPA-1a in 80%; HPA-5b in 15%) that is not present on maternal platelets can sensitize the mother to make antibodies that cross the placenta, coat the platelets which are then destroyed by the reticuloendothelial system and lead to serious bleeding, including intracranial haemorrhage. Alloimmune thrombocytopenia differs from HDN in that 50% of cases occur in the first pregnancy. Its incidence is approximately 1 in 1000–5000 births.

Thrombocytopenia can lead to serious, sometimes fatal, bleeding *in utero* or after birth. Treatment is unsatisfactory. Severe postnatal cases may be treated with a platelet transfusion that is negative for the relevant antigen. Antenatal treatment may be either maternal intravenous immunoglobulin or fetal transfusion with HPA-compatible platelets.

Coagulation

Standard tests need to be interpreted with caution in the neonate. The activated partial thromboplastin time (APTT) and prothrombin time (PT) are prolonged because of reduced levels of the vitamin K-dependent factors II, VII, IX and X, and return to normal at around 6 months. The thrombin time (TT) is comparable with adult values. Neonates have an increased risk of thrombosis. This is a result of physiologically low levels of inhibitors of coagulation and the use of indwelling vascular catheters. Antithrombin (AT) and protein C levels are approximately 60% of normal for the first 3 months. Homozygous protein C deficiency is associated with fulminant purpura fulminans in early life. Therapeutic protein C concentrates are now available. Homozygous AT deficiency usually presents later in childhood but arterial and venous thrombosis may also occur in the neonate.

Haemorrhagic disease of the newborn is discussed on page 297.

Haemolytic disease of the newborn

HDN is the result of ***red cell alloimmunization*** in which IgG antibodies passage from the maternal circulation across the placenta into the circulation of the fetus where they react with fetal red cells and lead to their destruction. Anti-D antibody is responsible for most cases of severe HDN although anti-c, anti-E, anti-K and a wide range of other antibodies are found in occasional cases (see Table 27.2). Although antibodies against the ABO blood group system are most frequent cause of HDN this is usually mild. Within the UK, approximately 500 fetuses develop haemolytic disease each year and approximately 30 of these cases are fatal.

Rh HDN

When an Rh D-negative (p. 339) woman has a pregnancy with an Rh D-positive fetus, Rh D-positive fetal red cells cross into the maternal circulation (especially at parturition and during the third trimester) and sensitize the mother to form anti-D. The mother could also be sensitized by a previous miscarriage, amniocentesis or other trauma to the placenta or by blood transfusion. Anti-D crosses the placenta to the fetus during the next pregnancy with an Rh D-positive fetus, coats the fetal red cells and results in reticuloendothelial destruction of these cells, causing anaemia and jaundice. If the father is heterozygous for D antigen, there is a 50% probability that the fetus will be D-positive. The fetal Rh D genotype can be established by polymerase chain reaction (PCR) analysis for the presence of Rh D in a maternal blood sample.

The main aim of management is to *prevent* anti-D antibody formation in Rh D-negative mothers. This can be achieved by the administration of small amounts of anti-D antibody which 'mop up' and destroy Rh D-positive fetal red cells before they can sensitize the immune system of the mother to produce anti-D.

Prevention of Rh immunization

At the time of booking, all pregnant women should have their ABO and Rh group determined and serum screened for antibodies at least twice during the pregnancy. All non-sensitized Rh D-negative women should be given at least 500 units (100 μg) of

anti-D at 28 and 34 weeks' gestation to reduce the risk of sensitization from fetomaternal haemorrhage. Fetal Rh D typing from DNA in maternal blood can be used before 28 weeks. If the fetus is Rh D-negative, no further anti-D prophylaxis is needed. In addition, at birth the babies of Rh D-negative women who do not have antibodies must have their cord blood grouped for ABO and Rh. If the baby's blood is Rh D-negative, the mother will require no further treatment. If the baby is Rh D-positive, prophylactic anti-D should be administered at a minimum dose of 500 units intramuscularly within 72 h of delivery. A ***Kleihauer test*** is performed. If the Kleihauer is positive many centres will perform flow cytometry for a more accurate estimate of the volume of feto-maternal haemorrhage (FMH). This uses differential staining to estimate the number of fetal cells in the maternal circulation (Fig. 28.5). The chance of developing antibodies is related to the number of fetal cells found. The dose of anti-D is increased if there is greater than 4 mL transplacental haemorrhage. Anti-D IgG (125 units) is given for each 1 mL of FMH greater than 4 mL.

Sensitizing episodes during pregnancy Anti-D IgG should be given to Rh D-negative women who have potentially sensitizing episodes during pregnancy: 250 units is given if the event occurs up to week 20 of gestation and 500 units thereafter, followed by a Kleihauer test. Potentially sensitizing events include therapeutic termination of pregnancy, spontaneous miscarriage after 12 weeks' gestation, ectopic pregnancy and invasive antenatal diagnostic procedures.

Treatment of established anti-D sensitization
If anti-D antibodies are detected during pregnancy they should be quantified at regular intervals. The strength of anti-D present in maternal serum is related to the clinical severity of HDN but this is also affected by such factors as the IgG subclass, rate of rise of antibody and past history. The development of haemolytic disease in the fetus can be assessed by velocimetry of the fetal middle cerebral artery by Doppler ultrasonography as increased velocities correlate with fetal anaemia (Fig. 28.6). If anaemia is detected, fetal blood sampling and intrauterine transfusion of irradiated Rh D-negative packed red cells may be indicated.

Clinical features of HDN

1 *Severe disease* Intrauterine death from hydrops fetalis (Fig. 28.7a).

2 *Moderate disease* The baby is born with anaemia and jaundice and may show pallor, tachycardia, oedema and hepatosplenomegaly. If the unconjugated bilirubin is not controlled and reaches levels exceeding 250 μmol/L, bile pigment deposition in the basal ganglia may lead to ***kernicterus***—central nervous system damage with generalized spasticity and possible subsequent mental deficiency, deafness and epilepsy. This problem becomes acute after birth as maternal clearance of fetal bilirubin ceases

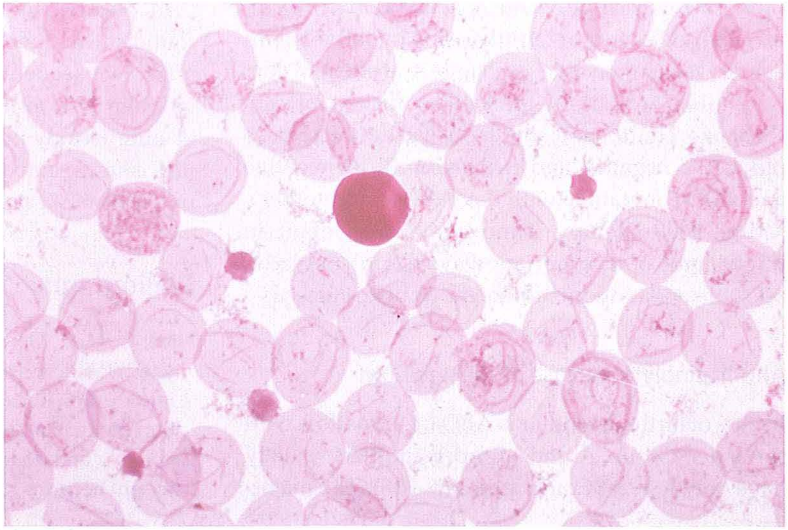


Fig. 28.5 Kleihauer test for fetal red cells; a deeply eosin-staining cell containing fetal haemoglobin is seen at the centre. Haemoglobin has been eluted from the other red cells by an incubation at acid pH and these appear as colourless ghosts.

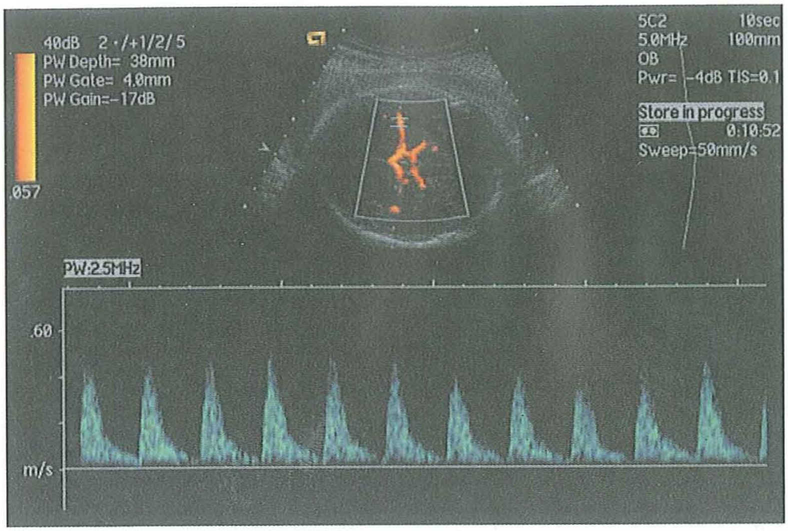


Fig. 28.6 Doppler ultrasonography of the circle of Willis in a fetus. The cursor is placed over the middle cerebral artery and an increased blood velocity correlates with anaemia. (From Kumar and Regan, 2005 with permission)

and conjugation of bilirubin by the neonatal liver has not yet reached full activity.

3 *Mild disease* Mild anaemia with or without jaundice.

Investigations will reveal variable anaemia with a high reticulocyte count; the baby is Rh D-positive, the direct antiglobulin test is positive and the serum bilirubin raised. In moderate and severe cases, many erythroblasts are seen in the blood film (Fig. 28.7b) and this is known as *erythroblastosis fetalis*.

Treatment

Exchange transfusion may be necessary; the indications for this include severe anaemia (Hb <10 g/dL at birth) and severe or rapidly rising hyperbilirubinaemia. More than one exchange transfusion may be required and 500 mL is usually sufficient for each exchange. The donor blood should be less than 5 days old, CMV negative and irradiated. Rh D-negative and ABO compatible with the baby's and mother's serum. Phototherapy (exposure of the infant to bright light of appropriate wavelength) degrades bilirubin and reduces the likelihood of kernicterus.

ABO haemolytic disease of the newborn

In 20% of births, a mother is ABO incompatible with the fetus. Group A and group B mothers usually have only IgM ABO antibodies (p. 338). The majority of cases of ABO HDN are caused by 'immune' IgG antibodies in group O mothers. Although 15% of pregnancies in white people involve a group O mother with a group A or group B fetus, most mothers do not produce IgG anti-A or anti-B and very few babies have severe enough haemolytic disease to require treatment. Exchange transfusions are needed in only 1 in 3000 infants. The mild course of ABO HDN is partly explained by the A and B antigens not being fully developed at birth and by partial neutralization of maternal IgG antibodies by A and B antigens on other cells, in the plasma and tissue fluids.

In contrast to Rh HDN, ABO disease may be found in the first pregnancy and may or may not affect subsequent pregnancies. The direct antiglobulin test on the infant's cells may be negative or only weakly positive. Examination of the blood film shows autoagglutination and spherocytosis, polychromasia and erythroblastosis.

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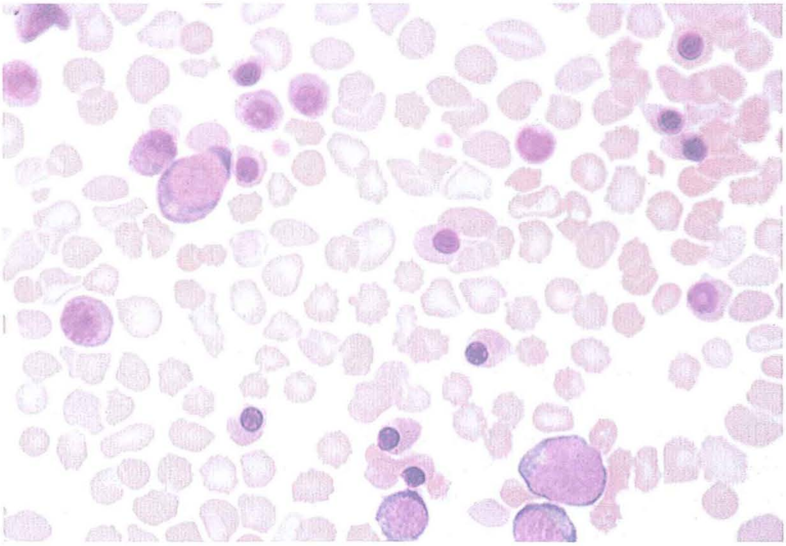
(a)



(b)

Fig. 28.7 **(a)** Ultrasound features of hydrops fetalis showing skin oedema, hepatomegaly and ascites. (From Kumar and Regan, 2005 with permission.) **(b)** Rh haemolytic disease of the newborn (erythroblastosis fetalis): peripheral blood film showing large numbers of erythroblasts, polychromasia and crenated cells.

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Cluster	Main cellular distribution	Name/comments/function/diagnostic value
CD158	NK cells and T-cell subsets	KIR
CD161	NK cells and some T cells	NKR-P1 family
CD162	Lymphocytes, neutrophils and monocytes	P-selectin glycoprotein ligand
CD163	Monocyte macrophage lineage	
CD164	Haemapoietic cells	MGC-24
CD165	Thymocytes, thymic epithelial cells	AD2/gp37
CD166	Thymic epithelial cells, activated T cells	CD6L
CD167	Epithelial cells	DDR1/receptor tyrosine kinase
CD168	Haemopoietic cells	RHAMM/hyaluronan-binding receptor
CD169	Macrophages	Sialoadhesin, siglec-1/cell–cell and cell–matrix interactions
CD171	Neurons, Epi (sub), lymphocyte (sub)	Homotypic and heterotypic cell adhesion
CD178	Activated T cells, neutrophils	FasL/cytokine
CD179a	Pro B and early pre B cells	VpreB/surrogate light chain
CD179b	Pro B and early pre B cells	λ5/surrogate light chain
CD184	Wide expression on haemopoietic cells	CXCR4/chemokine receptor/binds to SDF-1
CD233	Erythrocytes	Band 3/anion exchanger and attachment site for cytoskeleton
CD246	Few neural cells, subset of B-cell lymphoma	ALK/tyrosine kinase

ADAM, A disintegrin and metalloprotease; ALK, anaplastic lymphoma kinase; B-CLL, B-chronic lymphocytic leukaemia; c-ALL, common ($CD10^+$) acute lymphoblastic leukaemia; CD, cluster differentiation; C3dR, complement 3d receptor; EBV, Epstein–Barr virus; FcR, immunoglobulin Fc fragment receptor; FDC, follicular dendritic cell; G-CSFR, granulocyte colony-stimulating factor receptor; GM-CSFR, granulocyte–macrophage colony-stimulating factor; GP, glycoprotein; ICAM, intercellular adhesion molecule; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Ki, antibody to CD30; KIR, killer inhibitory receptors; LFA, leucocyte function-associated antigen; LPS, lipopolysaccharide; Mac-I, integrin molecule; MHC, major histocompatibility complex; NK, natural killer; PDGF, platelet-derived growth factor; SRCR, sheep red cell receptor; TGF, transforming growth factor; TNFR, tumour necrosis factor receptor; VLA, very late antigens; w, workshop.

Appendix 2

Normal values

	Males	Females	Males and females
Haemoglobin	13.5–17.5 g/dL	11.5–15.5 g/dL	
Red cells (erythrocytes)	$4.5–6.5 \times 10^{12}/L$	$3.9–5.6 \times 10^{12}/L$	
PCV (haematocrit)	40–52%	36–48%	
MCV			80–95 fL
MCH			27–34 pg
MCHC			20–35 g/dL
White cells (leucocytes)			
total			$4.0–11.0 \times 10^{9}/L$
neutrophils			$2.5–7.5 \times 10^{9}/L$
lymphocytes			$1.5–3.5 \times 10^{9}/L$
monocytes			$0.2–0.8 \times 10^{9}/L$
eosinophils			$0.04–0.44 \times 10^{9}/L$
basophils			$0.01–0.1 \times 10^{9}/L$
Platelets			$150–400 \times 10^{9}/L$
Red cell mass	30 ± 5 mL/kg	27 ± 5 mL/kg	
Plasma volume	45 ± 5 mL/kg	45 ± 5 mL/kg	
Serum iron			10–30 μmol/L
Total iron-binding capacity			40–75 μmol/L (2.0–4.0 g/L as transferrin)
Serum ferritin*	40–340 μg/L	14–150 μg/L	
Serum vitamin B_{12}*			160–925 ng/L
Serum folate*			3.0–15.0 μg/L
Red cell folate*			160–640 μg/L

* Normal ranges differ with different commercial kits.
MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume.

Appendix 3

Table 1 Proposed World Health Organization (WHO) classification of myeloid neoplasms.*

Myeloproliferative disease (MPD)
Chronic myelogenous leukaemia, Philadelphia chromosome (Ph1) [t(9;22)(qq34;q11), BCR/ABL]+
Chronic neutrophilic leukaemia
Chronic eosinophilic leukaemia/hypereosinophilic syndrome
Chronic idiopathic myelofibrosis
Polycythaemia vera
Essential thrombocythaemia
Myeloproliferative disease, unclassifiable
Myelodysplastic/myeloproliferative diseases
Chronic myelomonocytic leukaemia (CMML)
Atypical chronic myelogenous leukaemia (aCML)
Juvenile myelomonocytic leukaemia (JMML)
Myelodysplastic syndromes (MDS)
Refractory anaemia (RA)
with ringed sideroblasts (RARS)
without ringed sideroblasts
Refractory cytopenia (myelodysplastic syndrome) with multilineage dysplasia (RCMD)
Refractory anaemia (myelodysplastic syndrome) with excess blasts (RAEB)
5q-syndrome
Myelodysplastic syndrome, unclassifiable
Acute myeloid leukaemias (AML)*
Acute myeloid leukaemias with recurrent cytogenetic translocations
AML with t(8;21)(q22;q22), AML1(CBFα)/ETO
Acute promyelocytic leukaemia (AML with t(15;17)(q22;q11–12) and variants, PML/RAXα)
AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q11), CBFβ/MYH11X)
AML with 11q23 (MLL) abnormalities
Acute myeloid leukaemia with multilineage dysplasia
with prior myelodysplastic syndrome
without prior myelodysplastic syndrome
Acute myeloid leukaemia and myelodysplastic syndrome, therapy related
Alkylating agent related
Epipodophyllotoxin related (some may be lymphoid)
Other types

(*Continued*)

Table 1 Proposed World Health Organization (WHO) classification of myeloid neoplasms (*Continued*).*

Acute myeloid leukaemia not otherwise categorized
- AML minimally differentiated
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukaemia
- Acute monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryocytic leukaemia
- Acute basophilic leukaemia
- Acute panmyelosis with myelofiborisis

Acute biphenotypic leukaemias

* Only major disease categories are listed.

Table 2 Proposed World Health Organization (WHO) classification of lymphoid neoplasms.*

B-CELL NEOPLASMS

Precursor B-cell neoplasm
- *Precursor B-lymphoblastic leukaemia / lymphoma (precursor B-cell acute lymphoblastic leukaemia)*

Mature (peripheral) B-cell neoplasms**
- *B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma*
- B-cell prolymphocytic leukaemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone B-cell lymphoma (+/– villous lymphocytes)
- Hairy cell leukaemia
- *Plasma cell myeloma*/plasmacytoma
- *Extranodal marginal zone B-cell lymphoma of MALT type*
- Nodal marginal zone B-cell lymphoma (+/– monocytoid B cells)
- *Follicular lymphoma*
- *Mantle cell lymphoma*
- *Diffuse large B-cell lymphoma*
- Mediastinal large B-cell lymphoma
- Primary effusion lymphoma
- *Burkitt's lymphoma/Burkitt's cell leukaemia*

T AND NK-CELL NEOPLASMS

Precursor T-cell neoplasm
- *Precursor T-lymphoblastic lymphoma / leukaemia (precursor T-cell acute lymphoblastic leukaemia)*

Mature (peripheral) T-cell neoplasms**
- T-cell prolymphocytic leukaemia
- T-cell granular lymphocytic leukaemia
- Aggressive NK-cell leukaemia
- Adult T-cell lymphoma/leukaemia (HTLV1+)
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-type T-cell lymphoma
- Hepatosplenic $\gamma\delta$ T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma

(*Continued*)

Table 2 Proposed World Health Organization (WHO) classification of lymphoid neoplasms (*Continued*).*

Mycosis fungoides/Sézary syndrome
Anaplastic large-cell lymphoma, T/null cell, primary cutaneous type
Peripheral T-cell lymphoma, not otherwise characterized
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, T/null cell, primary systemic type
HODGKIN'S LYMPHOMA (HODGKIN'S DISEASE)
Nodular lymphocyte predominance Hodgkin's lymphoma
Classic Hodgkin's lymphoma
Nodular sclerosis Hodgkin's lymphoma (Grades 1 and 2)
Lymphocyte-rich classical Hodgkin's lymphoma
Mixed cellularity Hodgkin's lymphoma
Lymphocyte depletion Hodgkin's lymphoma

* More common entities are in italics.
** B- and T/NK-cell neoplasms are grouped according to major clinical presentations (predominantly disseminated/leukaemic, primary extranodal, predominantly nodal).

Table 3 Mast cell diseases.

Cutaneous mastocytosis
Systematic mast cell disease (+/– skin involvement)
Systematic mast cell disease with associated haematological disorder (+/– skin involvement)
Mast cell leukaemia/sarcoma

Table 4 Histiocytic and dendritic-cell neoplasms.

Macrophage/histiocytic neoplasm
Histiocytic sarcoma
Dendritic-cell neoplasms
Langerhans cell histiocytosis
Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma/tumour
Follicular dendritic cell sarcoma/tumour
Dendritic cell sarcoma, not otherwise specified (NOS)

Table 5 Plasma cell disorders: subtypes and variants.

Monoclonal gammopathy of undetermined significance (MGUS)
Plasma cell myeloma variants:
Indolent myeloma
Smoldering myeloma
Osteosclerotic myeloma (POEMS syndrome)
Plasma cell leukaemia
Non-secretory myeloma
Plasmacytoma variants:
Solitary plasmacytoma of bone
Extramedullary plasmacytoma

Table 6 Immunosecretory disorders (clinical manifestations of diverse lymphoid neoplasms).

Clinical syndrome	Underlying neoplasm
Waldenström's macroglobulinemia	Lymphoplasmacytic lymphoma
Heavy chain diseases (HCD):	
γ HCD	Lymphoplasmacytic lymphoma
α HCD	Extranodal marginal zone lymphoma (immunoproliferative small intestinal disorder)
μ HCD	B-cell chronic lymphocytic leukaemia
Immunoglobulin deposition diseases:	
Systemic light chain disease	Plasma cell myeloma, monoclonal gammopathy
Primary amyloidosis	Plasma cell myeloma, monoclonal gammopathy

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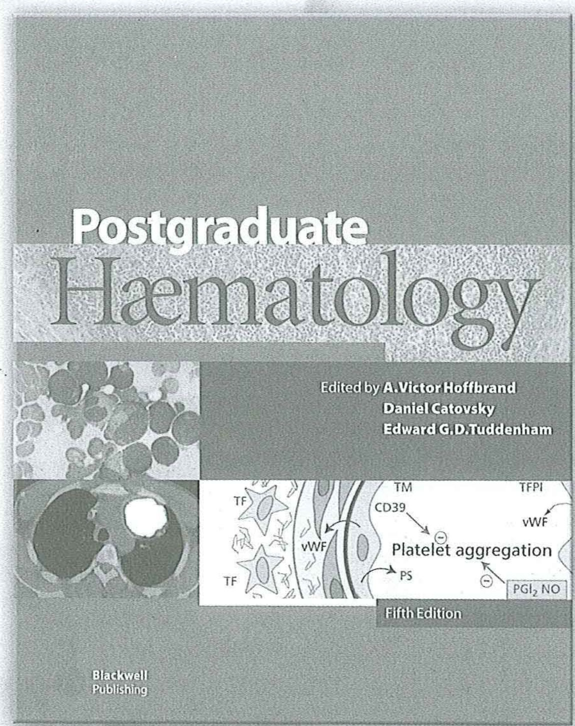

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